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FILE COVERS 1971 TO PATENT PUBLICATION DATE: 2 Feb 2006 (20060202/PD)

FILE LAST UPDATED: 2 Feb 2006 (20060202/ED)

HIGHEST GRANTED PATENT NUMBER: US6993790

HIGHEST APPLICATION PUBLICATION NUMBER: US2006026727

CA INDEXING IS CURRENT THROUGH 2 Feb 2006 (20060202/UPCA)

ISSUE CLASS FIELDS (/INCL) CURRENT THROUGH: 2 Feb 2006 (20060202/PD)

REVISED CLASS FIELDS (/NCL) LAST RELOADED: Dec 2005

USPTO MANUAL OF CLASSIFICATIONS THESAURUS ISSUE DATE: Dec 2005

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E1	2	WANG WEI KO/IN
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E6	3	WANG WEI MING/IN
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E11	1	WANG WEI SHENG/IN
E12	1	WANG WEI SHYANG/IN

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L1 12 "WANG WEI KUNG"/IN

L1 ANSWER 1 OF 12 USPTAFULL on STN
2005:57547 Apparatus monitoring signal in situ.
Wang, Wei-Kung, Taipei, TAIWAN, PROVINCE OF CHINA
US 2005049465 A1 20050303
APPLICATION: US 2004-924021 A1 20040823 (10)
PRIORITY: TW 2003-92123724 20030827
DOCUMENT TYPE: Utility; APPLICATION.

AB For repeatedly measuring signals from the same part of the tissue to monitor the blood composition, we use elastic membranes at upper and lower parts of the intruded tissue together with a cone-shaped guide. This will constrain the tissue in the same place when the signal guide is used for measuring the signals from the same part of tissue repeatedly. The signals can be from the aggregate of the designated composition with the other ingredients of the blood. For example, we may use the signals from the precursor of Glycated hemoglobin (HbA1c) to measure the concentration of blood glucose. For bio-secure purpose, the cone-shaped guide will be extended to cover tightly most part of the finger.

CLM What is claimed is:

1. An apparatus for monitoring a signal from a tissue in situ comprising: a tissue adaptor to fix the tissue at the same place during repeated measurements; a signal producer; and a signal analyzer; wherein the signal produced by the signal producer is interacted with the tissue and then be measured by the signal analyzer.

2. An apparatus as claimed in claim 1, wherein the said signal producer comprises a signal generator outside the tissue and interacts with the tissue to create the signal producer.

3. An apparatus as claimed in claim 1, wherein the said signal producer is in the tissue.

4. An apparatus as claimed in claim 1, wherein the tissue comprises an extrude shape.

5. An apparatus as claimed in claim 4, wherein the said extrude shape comprises a finger.

6. An apparatus as claimed in claim 1, wherein the said tissue adaptor comprises a soft pad.

7. An apparatus as claimed in claim 6, wherein the said soft pad is above the tissue.

8. An apparatus as claimed in claim 6, wherein the said soft pad is below the tissue.

9. An apparatus as claimed in claim 4, further comprising a cone shaped guide to confine the tissue.

10. An apparatus as claimed in claim 4, further comprising a concaved structure fit closely to the extruded tissue.

11. An apparatus as claimed in claim 2, wherein the said signal outside the tissue comprises electromagnetic wave.

12. An apparatus as claimed in claim 1, wherein the said signal producer comprises an ingredient in the tissue.

13. An apparatus as claimed in claim 12, wherein the said ingredient comprises an aggregate.

14. An apparatus as claimed in claim 13, wherein the said aggregate comprises glucose.

15. An apparatus as claimed in claim 13, wherein the said aggregate comprises hemoglobin.

16. An apparatus as claimed in claim 13, wherein the said aggregate comprises glucose and hemoglobin.

17. An apparatus as claimed in claim 1, wherein the said tissue adaptor comprises a flat area.

18. An apparatus as claimed in claim 1, wherein the said tissue adaptor comprises a spring.

19. An apparatus as claimed in claim 1, wherein the said tissue adaptor comprises an elastic membrane.

20. An apparatus as claimed in claim 1, wherein the said elastic

21. An apparatus as claimed in claim 1, wherein the said elastic membrane is below the tissue.
22. An apparatus for measuring a specific ingredient in blood comprises: a signal generator; and an analyzer to analyze the signal from the aggregate of said ingredient to determine the concentration of said ingredient.
23. An apparatus as claimed in claim 22, wherein the said blood comprises the signal generator.
24. An apparatus as claimed in claim 23, wherein said signal generator is generated from a signal from outside the blood and interacts with the blood to generate the signal.
25. An apparatus as claimed in claim 22, wherein the said specific ingredient comprises glucose.
26. An apparatus as claimed in claim 25, wherein the said glucose aggregate comprises hemoglobin.
27. An apparatus as claimed in claim 22, wherein the said signal from said aggregate comprises electromagnetic wave.
28. An apparatus as claimed in claim 22, wherein the said signal from the said aggregate comprises radiations.
29. An apparatus as claimed in claim 9, wherein the cone shaped guide is extended to cover tightly some part of the finger.

L1 ANSWER 2 OF 12 USPATFULL on STN

2004:268577 Mold-in method and apparatus.

Wang, Wei-Kung, Taipei, TAIWAN, PROVINCE OF CHINA
Wang, Gin-Chung, Taipei, TAIWAN, PROVINCE OF CHINA
US 2004210120 A1 20041021

APPLICATION: US 2004-752437 A1 20040106 (10)

PRIORITY: TW 2000-89104938 20000317

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention mainly relates to a method and apparatus for measuring the concentration of a solute in a solvent. Disclosed is an apparatus or method for determining the concentration of a solute in a solvent of a solution in a container having a time-varying volume by analyzing two signals received from the solution, comprising: measuring the quantity of the two received signals, converting the two signals into two electro-optical or electrical signals, performing a mathematical transformation on the two electro-optical or electrical signals, and determining the ratio of the transformation components of the two electro-optical or electrical signals. The present invention can be used in various applications of determining the ingredient concentration of a fluid, such as a gas or liquid. Particularly, the present invention finds applications in blood analysis in a human body for measuring, for example, the glucose, triglycerol, cholesterol, or oxyhemoglobin concentrations of the blood.

CLM What is claimed is:

1 An apparatus measuring the parameters in a volume with $V=V(t)$, where t is time; the apparatus comprising: two signal sources $A=A(t)$, $B=B(t)$ with $A(t)=B(t)K_0$, where $K_0>1$, $V(t)=B(t)K_1$, where K_0 , K_1 are stationary in a time interval t_0 , where t_0 is any real value; and detectors to measure the $B'(t)=B(t)+N_B(t)$ and the one assigned as $C'(t)=C(t)+N_A(t)$, where $C'(t)$ can be either $V'(t)=V(t)+N_V(t)$ or $A'(t)=A(t)+N_A(t)$, $N_B(t)$ is the noise of $B(t)$, $N_A(t)$ is the noise of $A(t)$, and $N_V(t)$ is the noise of $V(t)$ during the measurement time interval t_0 , wherein the measured signals $B'(t)$ and $C'(t)$ are transferred into electro optical signals and sent into a data processor to analyze either K_0 or K_1 .

2 An apparatus as claimed in claim 1 wherein $V=V(t)$ comprises: an additional property of $V(t)=K_2P(t)$, where $P(t)$ is the pressure in $V(t)$, K_2 is stationary in the time interval t_0 , and t_0 is any real number; and detectors to measure $P'(t)=P(t)+N_P(t)$, wherein $N_P(t)$ is the noise of $P(t)$ during the measurement time interval t_0 , to transfer the measured $B'(t)$ and $P'(t)$ into electro-optical signal and send the signal into a data processor to analyze K_2 .

3 An apparatus as claimed in claim 2, wherein the concentration of B is calculated from K_2 .

4 An apparatus as claimed in claim 2, wherein the elasticity of $V(t)$ is

5 An apparatus as claimed in claim 2, wherein the t_m is found at which $V(t_m)=V$ at maximum volume from $A(t)$ or $P(t)$.

6 An apparatus as claimed in claim 5, wherein the $V(t_m)$ is guiding the injection of an ingredient into V at t_m .

7 An apparatus as claimed in claim 1, wherein K_0 or K_1 is used to analyze the concentration of B .

8 An apparatus as claimed in claim 2, wherein K_2 is used to analyze the concentration of B .

9 An apparatus as claimed in claim 2, wherein one of the $P'(t)$ or $C'(t)$ is assigned as $E'(t)$, said data processor analyze the original data $B'(t)$ and $E'(t)$ by the following steps: performing a mathematical transformation T on both $E'(t)$ and $B'(t)$; estimating K_R from the following relation: $F_i[E'(t)]/F_i[B'(t)]=K_R$, $R:0$, or 1 , or 2 accordingly where F_i is the i th order component of the transformation T ; and determining the ratio of two signals $E(t)$ and $B(t)$ from the estimated K_R .

10 An apparatus as claimed in claim 9, wherein the mathematical transformation T is linear, said processor further performing the steps of: identifying and estimating $F_i[N_B(t)]$ by the noise around $F_i[E(t)]$; and determining the estimated K_R from the following relation: $\{F_i[E'(t)]-F_i[N_B(t)]\}/\{F_i[B'(t)]-F_i[N_B(t)]\}\approx K_R$.

11 An apparatus as claimed in claim 9, the processor further performing the step of: approximation K_R from the largest value of $F_i[E'(t)]-F_i[N_B(t)]$ for all kinds of linear transformation T and all possible orders of the transformation T , based on the following relation: $\{F_i[E'(t)]-F_i[N_B(t)]\}/\{F_i[B'(t)]-F_i[N_B(t)]\}\leq K_R$.

12 An apparatus as claimed in claim 9, wherein $E'(t)$ is statistically confident to be not noisy such that $N_E(t)\approx 0$.

$E'(t)=E(t)+N_E(t)\approx E(t)$, $B'(t)=B(t)+N_B(t)$, and $E(t)=K_R*B(t)$, said method comprising the steps of: performing a mathematical transformation T on both $E'(t)$ and $B'(t)$; estimating K_R from the following relation: $F_i[E'(t)]/F_i[B'(t)]\approx K$ where F_i is the i th order component of the transformation T and the position of $F_i[B'(t)]$ is identified by the noise around $F_i[E'(t)]$; and determining the ratio of two signals $E(t)$ and $B(t)$ from the estimated K_R .

13 An apparatus as claimed in claim 12, wherein the mathematical transformation T is linear, further comprising the steps of: identifying and estimating $F_i[N_B(t)]$ by the noise around $F_i[E(t)]$, and denoting the estimating of $F_i[N_B(t)]$ to be $F_i[N_E(t)]$; and estimating K_R from the following relation: $F_i[E(t)]/\{F_i[B'(t)]-F_i[N(t)]\}\approx K_R$.

14 An apparatus as claimed in claim 13, further comprising the steps of: approximation K_R from the largest value of $F_i[E'(t)]-F_i[N_B(t)]$ for all kinds of linear transformation T and all possible orders i of the transformation T , based on the following relation: $F_i[E(t)]/\{F_i[B'(t)]-F_i[N(t)]\}\leq K_R$.

15 An apparatus as claimed in claim 9, wherein the transformation T is Fourier transform.

16 An apparatus as claimed in claim 15, wherein the F_i is F_1 , the first harmonic of the Fourier transform.

17 An apparatus as claimed in claim 9, wherein the step for determining a ratio of two signals $E(t)$ and $B(t)$ based on two real signals $E'(t)$ and $B''(t)$ including noise $N_E(t)$ and $N_B(t)$, respectively, wherein: $E'(t)$ is a least noisy signal; $E'(t)=E(t)+N_E(t)$, $B'(t)=B(t)+N_B(t)$, and $E(t)=K_R*B(t)$, comprising the steps of: identifying the minimum of $B(t)$, $B'(t)_{\min}$, by $E'(t)$; and removing the static noise by $[B'(t)-B'(t)_{\min}]$.

18 An apparatus as claimed in claim 17, further comprising the steps of approximating K_R by using the following relation: Maximum of $[E(t)-E(t)_{\min}]/\text{Maximum of } [B(t)-B(t)_{\min}]=K_R$, where $E(t)_{\min}$ and $B(t)_{\min}$ are the minimum of $E(t)$ and $B(t)$, respectively.

19 An apparatus as claimed in claim 17, further comprising the steps of approximating K_R by using the following relation:

$$F_i[E(t) - E(t)_{\min}] / F_i[B'(t) - B(t)_{\min}] \approx K_R,$$

where both $E(t)$ and $B(t)$ are periodic and $E(t)_{\min}$ and $B(t)_{\min}$ are the minimum of $E(t)$ and $B(t)$, and F_i is the i^{th} order of a transformation.

20 An apparatus as claimed in claim 2, wherein the volume change in a periodic way.

21 An apparatus as claimed in claim 1, wherein the signal comprises induced signal.

22 An apparatus as claimed in claim 21, wherein the signal comprises and electromagnetic wave.

23 An apparatus as claimed in claim 21, wherein the induced signal comprises mechanical wave.

24 An apparatus as claimed in claim 1, wherein a signal source in the volume comprises a marker.

25 An apparatus as claimed in claim 1, wherein the volume comprises blood.

26 An apparatus as claimed in claim 1, wherein the volume comprises tissue.

27 An apparatus as claimed in claim 1, wherein a signal source comprises hemoglobin.

28 An apparatus as claimed in claim 1, wherein a signal source comprises uric acid.

29 An apparatus as claimed in claim 2 further comprises a pressure source for generating the volume change.

30 An apparatus as claimed in claim 1, wherein volume change in a periodic way.

31 An apparatus as claimed in claim 9, wherein the volume comprises blood, the blood pressure is measured by signal $E'(t)$.

32 An apparatus as claimed in claim 31, further comprising a instrument for measuring the blood flow $F'(t)$ in the volume, and means for determining K_p , which is an indicator of perfusion efficiency, based on the following relation: $F(t) = K_{pE}(t)$.

33 An apparatus as claimed in claim 6, further comprising an ingredient detector for injecting another ingredient in accordance with the result of the detector.

34 An apparatus as claimed in claim 33, wherein said ingredient comprises glucose and said another ingredient comprises insulin.

35 An apparatus as claimed in claim 1, wherein signal is transmitted through communication.

36 An apparatus as claimed in claim 1, wherein the volume is in a man-made system.

37 An apparatus as claimed in claim 1, wherein the signal source comprises DNA.

38 An apparatus as claimed in claim 1, wherein the signal source comprises RNA.

39 An apparatus as claimed in claim 1, wherein the signal source comprises protein.

40 An apparatus as claimed in claim 1, wherein the signal source comprises colored molecular.

41 An apparatus as claimed in claim 4, wherein the V is a pixie of $V(x, y, z)$, a much larger volume.

42 An apparatus as claimed in claim 41, wherein the $V(x, y, z)$ is compared with $V(x + \Delta x, y + \Delta y, z + \Delta z)$ in which Δx , Δy , Δz are the size of the pixie.

43 An apparatus as claimed in claim 41, wherein the $V(x, y, z)$ is compared

44 An apparatus as claimed in claim 1, wherein the signal source comprises glucose.

45 An apparatus as claimed in claim 1, wherein the signal source comprises cholesterol.

46 An apparatus as claimed in claim 1, wherein the signal source comprises triglycerol.

47 An apparatus as claimed in claim 1, wherein the signal source comprises enamation.

L1 ANSWER 3 OF 12 USPATFULL on STN

2004:207114 Apparatus for selectively moving hydrogen ions in aqueous solutions

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Bau, Jian-Guo, Taipei, TAIWAN, PROVINCE OF CHINA

Wei-Kung WANG (non-U.S. corporation)

US 2004159541 A1 20040819

APPLICATION: US 2004-780155 A1 20040217 (10)

PRIORITY: TW 2003-92103574 20030217

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB An apparatus selectively moving hydrogen ions in aqueous solution. Liquid water can form subcrystalline structure in a range of 5-20 molecules. The hydrogen ion can be among water molecules. Because the hydrogen ion acts as a hole in semiconductor and jumps forward in aqueous solutions, the hydrogen ion can be moved by a minimum electric field.

Since the major part of an organism is water, every organism can be considered as a container of aqueous solutions. The present invention provides the apparatus for selectively moving hydrogen ions in aqueous solution by low voltage and electrode with low impedance interface, thus for generating a specific electric current. To cope with the special requirements for measurement of electric signal and pH in vivo or reducing aqueous solution in viscosity, electrodes with special gel are disclosed in the apparatus. The apparatus can also be used in the heat therapy and deep acupuncture of tumor, since lactate may accumulate at regions of poor circulation and thus with a low pH. The medical apparatus manufactured accordingly has uses in tumor therapy, rehabilitation, weight loss, and so on.

CLM What is claimed is:

1. An apparatus for selectively moving hydrogen ions in aqueous solution comprising an electrical field generator which switches faster than 1 ms, and a low impedance electrical connection device which introduces said electrical field into a target.

2. The apparatus according to claim 1, further comprising a monitor of tracing a current signal not from the movement of said hydrogen ions.

3. The apparatus according to claim 1, wherein said aqueous solution is in an organism.

4. The apparatus according to claim 1, wherein a pH value is determined by a current supplied from said electrical field generator.

5. The apparatus according to claim 1, wherein said moving hydrogen ions is used to generate heat.

6. The apparatus according to claim 1, wherein said electrical field generator comprises generating step field.

7. The apparatus according to claim 1, wherein said electrical field generator comprises generating alternating field.

8. The apparatus according to claim 1, wherein said low impedance electrical connection device comprises a plurality of electrodes.

9. The apparatus according to claim 8, wherein said low impedance electrical connection device comprises at least one smaller-area electrode with a smaller area.

10. The apparatus according to claim 9, wherein said at least one smaller-area electrode is arranged into said target.

11. The apparatus according to claim 7, wherein said alternating field comprises a biphasic square wave.

12. The apparatus according to claim 1, wherein said electrical field

13. The apparatus according to claim 8, wherein said low impedance electrical connection device comprises a plurality of electrode pairs.
14. The apparatus according to claim 13, wherein the plurality of said electrode pairs generate electrical fields across said target.
15. The apparatus according to claim 1, wherein said target comprises a tumor.
16. The apparatus according to claim 1, wherein said target comprises a region of poor blood circulation.
17. The apparatus according to claim 2, wherein said monitor comprises a device for measuring the variation in said current.
18. The apparatus according to claim 2, wherein said monitor comprises an ultrasound-generating device.
19. The apparatus according to claim 14, wherein the plurality of said electrode pairs work at different times.
20. The apparatus according to claim 14, wherein the plurality of said electrode pairs generate electric fields that add in terms of vector at the target.
21. The apparatus according to claim 4, further comprising a calculating system for estimating the possibility of a cancer based on a concentration of the hydrogen ions.
22. The apparatus according to claim 21, wherein said estimation comprises the determination of positioning said cancer.
23. The apparatus according to claim 1, wherein said electric field generator comprises a magnetic field generator.
24. The apparatus according to claim 1, wherein said low impedance connection device comprises low pH ingredient.
25. The apparatus according to claim 24, wherein said low pH ingredient comprises an organic acid.
26. The apparatus according to claim 25, wherein said organic acid comprises a lactic acid.
27. The apparatus according to claim 25, wherein said organic acid comprises an acetic acid.
28. The apparatus according to claim 1, wherein said the moving of hydrogen ions is used to reduce the viscosity of said solution.
29. The apparatus according to claim 28, wherein said solution is in a small tube.
30. The apparatus according to claim 29, wherein said small tube is in an artificial machine.
31. The apparatus according to claim 30, wherein said small tube is in a microcirculation.
32. The apparatus according to claim 8, wherein the plurality of said electrodes comprise a temperature sensor.
33. A medium as interface to lower the impedance between a body and an electrode comprising a low pH solution.
34. The medium as claimed in claim 33, wherein said body comprises a biological fluid.
35. The medium as claimed in claim 33, wherein said low pH solution comprises an organic acid.
36. The medium as claimed in claim 35, wherein said organic acid comprises a lactic acid.
37. The medium as claimed in claim 35, wherein said organic acid comprises an acetic acid.

AB Disclosed is an apparatus for measuring the concentration of a specific ingredient in a solution. According to one embodiment of the present invention, said apparatus comprises: a signal collector for collecting a plurality of signals emitted from a target in a selected volume of the solution, and one of the signals being corresponding to the selected volume; detectors for detecting the signals; and beam splitters for splitting said signals and transmitting the signals to the detectors. The present invention provides an apparatus for effectively measuring concentration in-situ without the need of extracting the solution out of its original container.

CLM What is claimed is:

1. An apparatus for measuring the concentrations of (N-1) ingredients in a solution in-situ, wherein N is a natural number and $N \geq 2$, said apparatus comprising: a signal collector for collecting N signals from a target in a selected volume of the solution, one of said N signals being corresponding to said selected volume; means for detecting said N signals; and means for separating said N signals and transmitting said N separated signals to said detecting means.

2. The apparatus according to claim 1, wherein said N signals comprise at least one induced signal from said selected volume in response to an input signal.

3. The apparatus according to claim 2, wherein said input signal is in the form of electromagnetic wave.

4. The apparatus according to claim 1, wherein said signal collector comprises a plurality of cones for collecting said signals and/or for accommodating the transmission of said signals to said detecting means.

5. The apparatus according to claim 4, wherein said detecting means comprises a plurality of detectors respectively located at the tips of said plurality of cones.

6. The apparatus according to claim 1, wherein said separating means comprises a dichronic beam splitter.

7. The apparatus according to claim 1, wherein said separating means comprises N-1 beam splitters for separating said N signals.

8. The apparatus according to claim 7, wherein said signal collector comprises N cones for collecting said N signals.

9. The apparatus according to claim 5, wherein each of said plurality of cones comprises a lens for focusing the signal toward the corresponding detector.

10. The apparatus according to claim 4, wherein said plurality of cones comprise a highly reflective surface.

11. The apparatus according to claim 1, wherein said target comprises human tissue.

12. The apparatus according to claimed in claim 11, wherein said human tissue comprises a finger.

13. The apparatus according to claim 12, wherein said signal collector comprises an adapter located at the nail side of said finger for collecting said signals.

14. The apparatus according to claim 2, further comprising a signal guide for directing said input signal into said target.

15. The apparatus according to claim 14, wherein said signal guide directs said input signal into said target in said selected volume V at time t, and then said signal collector collects said signals from another selected volume V', which V' is the distribution of said target at time $t = t + \Delta t$.

16. The apparatus according to claim 15, wherein said target moves with a velocity V*, and said V' is a linear transition from V to $V + V^*t$.

17. The apparatus according to claim 16, wherein both said signal guide and signal collector respectively comprise a switch.

18. The apparatus according to claim 17, wherein the switch of said signal collector is open after a predetermined period of time when the switch of said signal guide is closed.

changed between open and close for a plurality of times.

20. The apparatus according to claim 14, wherein said target comprises human tissue.

21. The apparatus according to claim 20, wherein said human tissue comprises a finger.

22. The apparatus according to claim 21, wherein said signal guide is at the inner surface of said finger.

23. The apparatus according to claim 22, further comprising an envelope for securing said finger.

24. The apparatus according to claim 13, further comprising an envelope for securing said finger.

25. The apparatus according to claim 1, wherein said signal corresponding to said selected volume is a signal corresponding to the solvent of said solution.

26. The apparatus according to claim 1, wherein said signal corresponding to said selected volume is a signal corresponding to a marker with known concentration.

27. The apparatus according to claim 25, wherein said solvent comprises water.

28. A spectrophotometer, comprising: N cones for respectively collecting N signals; N detectors for respectively detecting wave lengths of said N signals; and N-1 dichronic beam splitters for respectively separating the spectrum of said N signals, wherein, N is a natural number and $N \geq 2$.

29. The spectrophotometer according to claim 28, wherein said N detectors are respectively located at the tips of said N cones.

30. The spectrophotometer according to claim 28, further comprises a lens.

31. The spectrophotometer according to claim 28, wherein said N cones respectively comprise a highly reflective surface.

32. The spectrophotometer according to claim 28, further comprising a monochrometer for selecting the wave lengths.

33. A finger adapter for spectroscopic studying, comprising a collector for collecting a light emitted from a specific volume of a finger of a human body, and for directing said light toward a spectrophotometer.

34. The finger adapter according to claim 33, further comprising a guide at the inner surface of said finger, for guiding the light toward a definite volume of said finger.

35. The finger adapter according to claim 34, further comprising an envelope for securing said finger.

36. The finger adapter according to claim 35, wherein both said collector and guide respectively comprise a switch.

37. The finger adapter according to claim 36, wherein the switch of said collector is open after a predetermined period of time when the switch of said guide is closed.

38. The apparatus according to claim 37, wherein said switches are changed between open and close for a plurality of times.

39. The finger adapter according to claim 37, wherein said specific volume is at a distance of $\Delta x = V \cdot \Delta t$ from said definite volume, in which V is the velocity of blood flow in said finger.

L1 ANSWER 5 OF 12 USPTAFULL on STN

2003:81254 Method and apparatus for monitoring and improving blood circulation by resonance.

Wang, Wei-Kung, No. 14, Sublane 3, Lane 61, Sec. 2, Yen Chiu Yuan Rd., Nan Kang District, Taipei, TAIWAN, PROVINCE OF CHINA
US 6537229 B1 20030325

APPLICATION: US 2000-604087 20000627 (9)

DOCUMENT TYPE: Utility; GRANTED.

AB The present invention relates to a method for improving the blood circulation of a human body by resonance, comprising the steps of: using

and coupling the energy through a coupling device to the circulatory system of the body by functionally connecting the coupling device to the body and then activating the energy generating device.

What is claimed is:

1. A method for improving blood circulation of a human body by resonance, comprising the steps of: (a) using an energy generating device to generate energy with a period synchronizing with the heartbeat of the body; and (b) coupling the energy through a coupling device to the circulatory system of the body by functionally connecting the coupling device to the body and then activating the energy generating device.
2. The method as claimed in claim 1, wherein the energy generating device is an artificial heart and step (b) further comprises connecting tubes to an artery of the body.
3. The method as claimed in claim 1, wherein the energy generating device comprises a motor with a speed synchronized with the heartbeat of the body.
4. The method as claimed in claim 3, wherein the step (b) further comprises: (c) providing an impedance matched mattress filled with excited fluid; (d) exciting the natural frequency of the mattress by the motor; and (e) treatment by subjecting the body to the mattress.
5. The method as claimed in claim 3, wherein the heartbeat is measured by an EKG.
6. The method as claimed in claim 3, wherein the coupling device comprises a cup to be fitted into an extrusive part of the body, and the energy generating device comprises a suction pump to be turned on to suck on the extrusive part.
7. The method as claimed in claim 3, wherein the coupling device comprises a disk with a number of extrusive parts. The disk is driven by a motor to rotate at a rate identical to the rate of the heartbeat which is selected according to the designated meridian for application.
8. The method as claimed in claim 3, the coupling channel comprises an exercise machine moving with the rate of the heartbeat as meter and may be fine-tuned according to the beat requirement of the limbs.
9. The method as claimed in claim 1, wherein said energy generating device comprises a music playing machine, and said coupling device comprises a dancing or exercising machine.
10. The method as claimed in claim 1, wherein said energy generated by said energy generating device is in the form of one selected from the group of sound, light, electricity and pressure, and said coupling device comprises either a sensor organ or an artificial sensor device.
11. A method for improving blood circulation of a human body by resonance, comprising the steps of: (a) using an energy generating device to generate energy resonating with the body; (b) coupling the energy through a coupling device to the circulatory system of the body by functionally connecting the coupling device to the body and then activating the energy generating device; and (c) further comprising the step of feedback-control of the energy generating device by a monitoring device.
12. The method as claimed in claim 11, wherein the monitoring device comprises a pulse analysis machine to analyze the pulse spectrum of the body.
13. The method as claimed in claim 11, wherein the monitoring device comprises a heart rate variation machine to analyze the HRV of the body.
14. The method as claimed in claim 11, further comprising the following steps performed by the monitoring device: (a) measuring pressure waves formed in an artery of the body and producing an electrical pulse representing a blood pressure pulse; and (b) calculating the amplitude and the phase of harmonic components of the electrical pulse by applying Fourier transformation theory to the harmonics of the electrical pulse.
15. The method as claimed in claim 14, wherein the step of measuring the pressure wave comprises the step of sensing the blood pressure pulse in an artery of the body with a pressure transducer.
16. The method as claimed in claim 14, wherein the step of measuring the pressure waves comprises directing a light beam toward the blood in the artery and then measuring absorption rate of a blood component by a photo detector.

17. The method as claimed in claim 16, wherein the blood component comprises water.
18. The method as claimed in claim 16, wherein the blood component comprises hemoglobin.
19. The method as claimed in claim 14, wherein the step (b) further comprises displaying a standard deviation of the measured harmonic component.
20. The method as claimed in claim 19, wherein the step (b) further comprises using a coefficient of variance, which equals a standard deviation divided by the mean, as an indicator.
21. The method as claimed in claim 14, wherein the fourth harmonic is used to monitor the health condition of the lung and lung meridian of the body.
22. The method as claimed in claim 14, wherein the sixth harmonic is used to monitor the health condition of the gall-bladder meridian of the body.
23. The method as claimed in claim 14, wherein the first harmonic is used to monitor the health condition of the liver meridian which includes the brain stem.
24. An apparatus for improving the blood circulation of a human body by resonance comprising an energy generating device for generating an energy with a period synchronizing with the heartbeat of the body, and coupling device for delivering the energy to the circulatory system of the body.
25. The apparatus as claimed in claim 24, wherein the energy generating device comprises a motor rotating at a rate identical to that of the heart rate, and the coupling device comprises a disk attached to a motor with a number of extrusive parts.
26. The apparatus as claimed in claim 25, wherein the number of extrusive parts is selected according to the meridian to be treated.
27. The apparatus as claimed in claim 24, the energy generating device comprises a heartbeat detector.
28. The apparatus as claimed in claim 24, the energy generating device comprises an electrical signal generator and a coupling device is functionally connected to a part of the body.
29. The apparatus as claimed in claim 28, the electrical signal comprises a weak stimulus which is delivered during the appearance of systolic pressure in the body and will cause a muscular twitch.
30. The apparatus as claimed in claim 28, the electrical signal comprises a strong stimulus which is delivered during the appearance of diastolic pressure in the body and will not cause any muscular twitch.
31. The apparatus as claimed in claim 24, wherein the coupling device is further coupled to an acupuncture point.
32. The apparatus as claimed in claim 24, wherein the energy generating device comprises a suction pump, and the coupling device comprises a cup for fitting onto an extrusive part of the body.
33. The apparatus as claimed in claim 32, wherein the extrusive part of the body comprises the penis of the body.
34. The apparatus as claimed in claim 32, wherein the extrusive part of the body comprises the breast of the body.
35. The apparatus as claimed in claim 24, wherein said energy generating device comprises a measuring instrument with a controllable meter to be synchronized with the heartbeat of the body, and said coupling device comprises a dancing of exercising machine.
36. An apparatus for improving the blood circulation of a human body by resonance comprising an energy generating device for generating an energy resonant with the body, and coupling device for delivering the energy to the circulatory system of the body, wherein the coupling device further comprises a mattress filled with fluid and a frequency tuning device and the energy generating device further comprises a motor driven device for sending energy to the mattress so as to generate resonant waves in the mattress.

device further comprises a pressure controlling device.

38. The apparatus as claimed in claim 36, wherein the frequency tuning device further comprises a shape changing device.

L1 ANSWER 6 OF 12 USPTAFULL on STN

2003:44042 Biosecure method and device.

Wang, Wei-Kung, Taipei, TAIWAN, PROVINCE OF CHINA

US 2003031347 A1 20030213

APPLICATION: US 2002-207610 A1 20020729 (10)

PRIORITY: TW 2001-90119107 20010802

DOCUMENT TYPE: Utility; APPLICATION.

AB Disclosed is a biosecure method and device for identifying a person. According to one embodiment of the present invention, said device comprises: a light source for directing a light beam to a part of the person's body; a detector for measuring the light from the part of the person's body and converting the light intensity into electrical signals of a spectrum; and a spectrum analyzer and comparator for analyzing the spectrum and comparing the spectrum with stored spectrum for the person. The present invention provides a biosecure system, which is simple, cost-effective and hard to be faked.

CLM What is claimed is:

1. A biosecure method for identifying a person, comprising: directing a light beam to a part of the person's body; analyzing a light spectrum generated therefrom; and comparing analysis result of the spectrum with stored data for the person.

2. The biosecure method according to claim 1, wherein said spectrum comprises transmitted light spectrum.

3. The biosecure method according to claim 1, wherein said spectrum comprises scattered light spectrum.

4. The biosecure method according to claim 1, wherein said spectrum comprises changes with respect to time.

5. The biosecure method according to claim 1, wherein said spectrum comprises infrared spectrum.

6. The biosecure method according to claim 1, wherein said light beam is substantially a single-wavelength one.

7. The biosecure method according to claim 1, wherein said part of the person's body is a finger of that person.

8. The biosecure method according to claim 1, further comprising the step of: attaching an envelope to the part of the person's body.

9. The biosecure method according to claim 1, further comprising the step of: directing the light beam to another one part or a plurality of parts of the person's body to generate additional light spectrum for subsequent analysis and comparison.

10. The biosecure method according to claim 9, wherein said step of directing the light beam are performed for a plurality of times and arranged in a particularly designed sequence.

11. The biosecure method according to claim 1, further comprising the step of: updating the stored data by the analysis result, if the person is correctly identified.

12. A biosecure device for identifying a person, comprising: a light source for directing a light beam to a part of the person's body; a detector for measuring the light from the part of the person's body and converting the light intensity into electrical signals of a spectrum; and a spectrum analyzer and comparator for analyzing the spectrum and comparing the spectrum with stored spectrum for the person.

13. The biosecure device according to claim 12, said light beam is substantially a single-wavelength one.

14. The biosecure device according to claim 12, wherein said light source comprises an LED.

15. The biosecure device according to claim 12, wherein said part of the person's body is further attached with an envelope.

16. A personal electronic apparatus comprising the biosecure device according to claim 12.

17. The personal electronic apparatus according to claim 16, wherein the

18. The personal electronic apparatus according to claim 16, wherein the personal electronic apparatus is a computer.

19. The biosecure device according to claim 12, wherein the biosecure device is designed in the way of lock-and-key.

20. The biosecure device according to claim 12, wherein said spectrum comprises changes with respect to time.

21. The biosecure device according to claim 20, wherein said changes are analyzed by way of frequency analysis.

22. The biosecure device according to claim 21, wherein said frequency analysis comprises harmonic analysis.

L1 ANSWER 7 OF 12 USPTAFULL on STN

2002:280001 Detection of dengue virus.

Wang, Wei-Kung, Taipei, TAIWAN, PROVINCE OF CHINA

US 2002155435 A1 20021024

APPLICATION: US 2002-85944 A1 20020228 (10)

PRIORITY: US 2001-272535P 20010301 (60)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to a pair of dengue virus-specific primers for use in a reverse transcriptase-polymerase chain reaction to detect dengue virus.

CLM What is claimed is:

1. A method for detecting dengue virus comprising: obtaining a sample which is suspected of containing dengue virus RNA; performing a reverse transcriptase-polymerase chain reaction on the sample with a first dengue virus-specific primer and a second dengue virus-specific primer to amplify the dengue virus RNA, if present, wherein the first dengue virus-specific primer is 18 to 28 nucleotides in length and includes at least 18 consecutive nucleotides of SEQ ID NO: 1, and the second dengue virus-specific primer is 18 to 28 nucleotides in length and includes at least 18 consecutive nucleosides of SEQ ID NO: 2; and detecting the amplification product as an indication of presence of dengue virus in the sample.

2. The method of claim 1, wherein the first dengue virus-specific primer is 18 to 23 nucleotides in length.

3. The method of claim 1, wherein the first dengue virus-specific primer is the nucleotide sequence of SEQ ID NO: 1.

4. The method of claim 1, wherein the second dengue virus-specific primer is 18 to 23 nucleotides in length.

5. The method of claim 1, wherein the second dengue virus-specific primer is the nucleotide sequence of SEQ ID NO: 2.

6. The method of claim 2, wherein the second dengue virus-specific primer is 18 to 23 nucleotides in length.

7. The method of claim 3, wherein the second dengue virus-specific primer is the nucleotide sequence of SEQ ID NO: 2

8. A method for quantitating dengue virus comprising: obtaining a sample which is suspected of containing dengue virus RNA, and mixing it with a known amount of a competitor nucleic acid; performing a reverse transcriptase-polymerase chain reaction on the sample and the competitor nucleic acid with a first dengue virus-specific primer and a second dengue virus-specific primer to amplify the dengue virus RNA, if present, and the competitor nucleic acid, wherein the first dengue virus-specific primer is 18 to 28 nucleotides in length and includes at least 18 nucleotides of SEQ ID NO: 1, and the second dengue virus-specific primer is 18 to 28 nucleotides in length and includes at least 18 nucleosides of SEQ ID NO: 2; and comparing the amounts of the amplification product of the dengue virus RNA, if present, to the amplification product of the competitor nucleic acid to quantitate the dengue virus RNA in the sample.

9. The method of claim 8, wherein the first dengue virus-specific primer is 18 to 23 nucleotides in length.

10. The method of claim 8, wherein the first dengue virus-specific primer is the nucleotide sequence of SEQ ID NO: 1.

11. The method of claim 8, wherein the second dengue virus-specific primer is 18 to 23 nucleotides in length.

12. The method of claim 8, wherein the second dengue virus-specific primer is the nucleotide sequence of SEQ ID NO: 2.
13. The method of claim 9, wherein the second dengue virus-specific primer is 18 to 23 nucleotides in length.
14. The method of claim 10, wherein the second dengue virus-specific primer is the nucleotide sequence of SEQ ID NO: 2.
15. A kit for detecting dengue virus comprising: A first dengue virus-specific primer, which is 18 to 28 nucleotides in length and includes at least 18 nucleotides of SEQ ID NO: 1; and A second dengue virus-specific primer, which is 18 to 28 nucleotides in length and includes at least 18 nucleotides of SEQ ID NO: 2.
16. The kit of claim 15, further comprising a known amount of a competitor nucleic acid with length detectably different from the dengue virus RNA.
17. The kit of claim 15, wherein the first dengue virus-specific primer is 18 to 23 nucleotides in length.
18. The kit of claim 15, wherein the first dengue virus-specific primer is the nucleotide sequence of SEQ ID NO: 1.
19. The kit of claim 15, wherein the second dengue virus-specific primer is 18 to 23 nucleotides in length.
20. The kit of claim 15, wherein the second dengue virus-specific primer is the nucleotide sequence of SEQ ID NO: 2.
21. The kit of claim 17, wherein the second dengue virus-specific primer is 18 to 23 nucleotides in length.
22. The kit of claim 18, wherein the second dengue virus-specific primer is the nucleotide sequence of SEQ ID NO: 2.
23. A nucleic acid, which is 18 to 28 nucleotides in length and includes at least 18 consecutive nucleotides of SEQ ID NO: 1.
24. The nucleic acid of claim 23, wherein the nucleic acid is 18 to 23 nucleotides in length and includes at least 18 consecutive nucleotides of SEQ ID NO: 1.
25. The nucleic acid of claim 23, wherein the nucleic acid is the nucleotide sequence of SEQ ID NO: 1.
26. A nucleic acid, which is 18 to 28 nucleotides in length and includes at least 18 consecutive nucleotides of SEQ ID NO: 2.
27. The nucleic acid of claim 26, wherein the nucleic acid is 18 to 23 nucleotides in length and includes at least 18 consecutive nucleotides of SEQ ID NO: 2.
28. The nucleic acid of claim 26, wherein the nucleic acid is the nucleotide sequence of SEQ ID NO: 2.
29. An isolated nucleic acid comprising a fragment of a dengue viral genome or a DNA copy thereof, wherein the fragment includes: a first sequence that is complementary or identical to at least 18 consecutive nucleotides of SEQ ID NO: 1; a second sequence that is complementary or identical to at least 18 consecutive nucleotides of SEQ ID NO: 2; and a non-naturally occurring deletion or insertion, the deletion or insertion occurring in a region of the fragment flanked by the first and the second sequence.
30. The nucleic acid of claim 29, wherein the first sequence is complementary or identical to SEQ ID NO: 1 and the second sequence that is complementary or identical to SEQ ID NO: 2.

L1 ANSWER 8 OF 12 USPTAFULL on STN

2001:161123 Mold-in method and apparatus.

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Wang, Gin-Chung, Taipei, Taiwan, Province of China

US 2001023391 A1 20010920

APPLICATION: US 2001-766237 A1 20010119 (9)

PRIORITY: TW 2000-89104938 20000317

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention mainly relates to a method and apparatus for measuring the concentration of a solute in a solvent. Disclosed is an

solvent of a solution in a container having a time-varying volume by analyzing two signals received from the solution, comprising: measuring the quantity of the two received signals, converting the two signals into two electro-optical or electrical signals, performing a mathematical transformation on the two electro-optical or electrical signals, and determining the ratio of the transformation components of the two electro-optical or electrical signals. The present invention can be used in various applications of determining the ingredient concentration of a fluid, such as a gas or liquid. Particularly, the present invention finds applications in blood analysis in a human body for measuring, for example, the glucose, triglycerol, cholesterol, or oxyhemoglobin concentrations of the blood.

CLM What is claimed is:

1. A method (Mold-In strong) for determining a ratio of two signals $A(t)$ and $B(t)$ based on two real signals $A'(t)$ and $B'(t)$ including noise $N_A(t)$ and $N_B(t)$, respectively, wherein:

$$N_A(t) \approx N_B(t), \quad A'(t) = A(t) + N_A(t),$$

$$B'(t) = B(t) + N_B(t), \quad \text{and} \quad A(t) = K_0 * B(t), \quad K_0 > 1, \quad \text{said method}$$

comprising the steps of: (a) performing a mathematical transformation T on both $A'(t)$ and $B'(t)$; and (b) estimating K_0 from the following

$$\text{relation: } F_i[A'(t)]/F_i[B'(t)] \approx K_0 \text{ phd } 0, \quad \text{where } F_i$$

is the i^{th} order component of the transformation T ; and (c)

determining the ratio of two signals $A(t)$ and $B(t)$ from the estimated

$$K_0.$$

2. The method as claimed in claim 1, wherein the mathematical transformation T is linear, said method further comprising the steps of:

(d) identifying and estimating $F_i[N_B(t)]$ by the noise around

$F_i[A(t)]$; and (e) determining the estimated K_0 from the following

$$\text{relation: } \{F_i[A'(t)] - F_i[N_B(t)]\} / \{F_i[B'(t)] -$$

$$F_i[N_B(t)]\} \approx K_0.$$

3. The method as claimed in claim 2, further comprising the step of:

(f) approximating K_0 from the largest value of $F_i[A'(t)]/F_i$

$[B'(t)]$ for all kinds of linear transformation T and all possible orders

i of the transformation T , based on the following relation:

$$\{F_i[A'(t)] - F_i[N_B(t)]\} / \{F_i[B'(t)] -$$

$$F_i[N_B(t)]\} \leq K_0.$$

4. A method (Mold-In medium) for determining a ratio of two signals $A(t)$ and $B(t)$ based on two real signals $A'(t)$ and $B'(t)$ including noise

$N_A(t)$ and $N_B(t)$, respectively, wherein: $A'(t)$ is

statistically confident to be not noisy such that $N_A(t) \approx 0$,

$$A'(t) = A(t) + N_A(t) \approx A(t), \quad B'(t) = B(t) + N_B(t), \quad \text{and}$$

$$A(t) = K_0 * B(t), \quad \text{said method comprising the steps of: (a) performing}$$

a mathematical transformation T on both $A'(t)$ and $B'(t)$; and (b)

estimating K_0 from the following relation:

$$F_i[A(t)]/F_i[B'(t)] \approx K_0, \quad \text{where } F_i \text{ is the}$$

i^{th} order component of the transformation T and the position of

$F_i[B'(t)]$ is identified by the noise around $F_i[A(t)]$; and

(c) determining the ratio of two signals $A(t)$ and $B(t)$ from the

estimated K_0 .

5. The method as claimed in claim 4, wherein the mathematical

transformation T is linear, further comprising the steps of: (d)

identifying and estimating $F_i[N_B(t)]$ by the noise around F_i

$[A(t)]$, and denoting the estimation of $F_i[N_B(t)]$ to be F_i

$[N(t)]$; and (e) estimating K_0 from the following relation:

$$F_i[A(t)] / \{F_i[B'(t)] - F_i[N(t)]\} \approx K_0.$$

6. The method as claimed in claim 5, further comprising the step of:

(e) approximating K_0 from the largest value of K_0 for all

kinds of linear transformation T and all possible orders i of the

transformation T , based on the following relation:

$$F_i[A(t)] / \{F_i[B'(t)] - F_i[N(t)]\} \leq K_0.$$

7. The method as claimed in claim 2 or 5, wherein the transformation T is a Fourier transform.

8. The method as claimed in claim 7, wherein the F_i is F_1 , the first 15 harmonic of the Fourier transform.

9. A method (Mold-In weak) for determining a ratio of two signals $A(t)$ and $B(t)$ based on two real signals $A'(t)$ and $B'(t)$ including noise

$N_A(t)$ and $N_B(t)$, respectively, wherein: $A'(t)$ is a less noisy

signal; $A'(t) = A(t) + N_A(t)$, $B'(t) = B(t) + N_B(t)$, and

$$A(t) = K_0 * B(t), \quad \text{comprising the steps of: (a) identifying the}$$

minimum of $B'(t)$, $B'(t)_{\min}$, by $A'(t)$; and (b) removing the static

noise by $[B'(t) - B'(t)_{\min}]$.

10. The method as claimed in claim 9, further comprising the step of approximating K_0 by using the following relation: Maximum of $[A(t) - A(t)_{\min}] / \text{Maximum of } [B(t) - B(t)_{\min}] \approx K_0$, where $A(t)_{\min}$ and $B(t)_{\min}$ are the minimum of $A(t)$ and $B(t)$, respectively.

11. The method as claimed in claim 9, further comprising the step of approximating K_0 by using the following relation:

$F_1[A(t) - A(t)_{\min}] / F_1[B(t) - B(t)_{\min}] \approx K_0$, where both $A(t)$ and $B(t)$ are periodic and $A(t)_{\min}$ and $B(t)_{\min}$ are the minimum of $A(t)$ and $B(t)$, and F_1 is the 1st order harmonic of Fourier transform.

12. An apparatus for determining the concentration of a solute in a solvent of a solution in a container having a time-varying volume by analyzing two signals received from the solution, comprising: a detector for measuring the quantity of the two received signals; a signal converter for converting the two signals into two electro-optical signals; and means for determining a ratio of the two electro-optical signals by performing the method as claimed in claims 1, 4 or 9.

13. The apparatus as claimed in claim 12, wherein the container having a time-varying volume is blood vessel in a human body and the solution is blood of the body.

14. The apparatus as claimed in claim 12, wherein both the two received signals are induced by directing an input signal into the solution.

15. The apparatus as claimed in claim 12, wherein the volume changes in a periodic way.

16. The apparatus as claimed in claim 14, wherein the input signal is an electromagnetic wave.

17. The apparatus as claimed in claim 12, wherein an ingredient of the solution is a marker.

18. The apparatus as claimed in claim 13, wherein the blood vessel is in an extrusive part of the human body.

19. The apparatus as claimed in claim 18, wherein the extrusive part of the human body is a finger.

20. The apparatus as claimed in claim 12, wherein the solute comprises glucose.

21. The apparatus as claimed in claim 12, wherein the solute comprises uric acid.

22. An apparatus for measuring the concentration of a solute in a solvent of a solution in a container having a time-varying volume by analyzing two signals received from the solution, comprising: a pressure source for generating the volume change of the time-varying volume; a detector for detecting the two received signal; a signal converter for converting the two received signals into two electrical signals; and means for determining a ratio of the two electrical signals by performing the method as claimed in claims 1, 4 or 9.

23. The apparatus as claimed in claim 22, wherein the pressure source is controlled to generate the effective volume in a periodic way.

24. The apparatus as claimed in claim 23, wherein the periodicity of generating the effective volume follows a trigonometric function.

25. An apparatus for measuring the blood pressure variation $[P(t) - P(t)_{\text{diastolic}}]$ in a human body by a marker signal $B'(t)$ in the blood of the body, comprising: a detector for measuring the marker signal $B'(t)$; and a data processing unit determining the $[P(t) - P(t)_{\text{diastolic}}]$ based on $[B'(t) - B'_{\min}(t)]$, where: $P(t)$ is blood pressure as function of time, $P(t)_{\text{diastolic}}$ is diastolic or minimum of the $P(t)$, and $B'_{\min}(t)$ is the minimum of the marker signal $B'(t)$.

26. The apparatus as claimed in claim 25, further comprising a Laser Doppler instrument for measuring the blood flow velocity $D(t)$ into tissue, and means for determining K_1 , which is an indicator of perfusion efficiency, based on the following relation: $[D_{\max}(t) - D_{\min}(t)] / [P_{\text{systolic}}(t) - P_{\text{diastolic}}(t)] = K_1$, where, $P_{\text{systolic}}(t)$ is systolic or maximum of $P(t)$, $D_{\max}(t)$ is the maximum of $D(t)$, and $D_{\min}(t)$ is the minimum of $D(t)$.

27. The apparatus as claimed in claim 25, further comprising an injection device for injecting a drug during the period of the P(t)_{systolic}.
28. The apparatus as claimed in claim 27, further comprising a blood ingredient detector for injecting the drug in accordance with the result of the detector.
29. The apparatus as claimed in claim 28, wherein said blood ingredient comprises glucose and said drug comprises insulin.
30. The apparatus as claimed in claim 12, wherein information related to the concentration is transmitted through telephone communication.
31. The apparatus as claimed in claim 12, wherein the container is in a micro-electro mechanic system (MEMS).
32. The apparatus as claimed in claim 17, wherein the marker comprises a solvent.
33. The apparatus as claimed in claim 32, wherein the solvent comprises water.

L1 ANSWER 9 OF 12 USPTAFULL on STN

2000:58877 Method and apparatus for detecting charged particles in an aqueous solution.

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Wang, Wei Kung, Taipei, Taiwan, Province of China (non-U.S. individual)
US 6061585 20000509

APPLICATION: US 1997-901532 19970728 (8)

DOCUMENT TYPE: Utility; Granted.

- AB A method of detecting the charged particles in an aqueous solution, including immersing one of two electrodes in the aqueous solution to provide a conductive path between the aqueous solution and the other electrode; applying a stepping potential through the electrodes to measure an impulse current through the aqueous solution; and comparing the measured impulse current with an impulse current of a reference solution having known ingredients to determine if the two solutions are similar.
- CLM What is claimed is:
1. A method for detecting charged particles in an aqueous solution comprising the steps of: (a) immersing a first electrode of an electrode pair in the aqueous solution to provide a conductive path between the aqueous solution and a second electrode of the electrode pair, wherein the second electrode is not immersed in the aqueous solution; (b) applying a stepping potential through said electrode pair to measure an impulse current through the aqueous solution; and (c) comparing the impulse current with an impulse current of a reference solution having known ingredients to determine if the aqueous and the reference solutions are similar.
 2. The method of claim 1, wherein the aqueous solution is within a biological body.
 3. The method of claim 2, wherein one of said electrode pair includes a needle to penetrate tissue of the biological body to detect charged particles in the aqueous solution within the tissue.
 4. The method of claim 2, wherein one of the known ingredients is lactate.

L1 ANSWER 10 OF 12 USPTAFULL on STN

1998:29870 Method and apparatus for diagnosing and monitoring the circulation of blood.

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US 5730138 19980324

APPLICATION: US 1995-520820 19950830 (8)

DOCUMENT TYPE: Utility; Granted.

- AB A method and apparatus for diagnosing the circulation of blood. A pressure measurement instrument is provided to measure the waveform of the blood pressure in an artery of a patient. An analyzer is employed to analyze the frequency components of the blood pressure wave in order to compare the pattern of each resonance component to the pattern of a normal blood pressure wave in order to determine whether the blood distribution of the patient is off-balance. This off-balance can be diagnosed from the Chinese medicine principles by relating each harmonic in the blood pressure wave to the corresponding meridian which includes a specific organ. If a specific problem or disorder is identified, the

in the specific harmonic component in the blood pressure wave. This method can also be a valuable medical tool in the development of new treatments or drugs. The analyzer includes a device for analyzing the amplitude and the phase of the resonant frequencies in the blood circulation of the body, and includes a transducer attached to or closely adjacent to the surface of an artery of the body of the patient. The analyzer includes a computer for analyzing the frequency spectrum of blood pressure in the artery for determining characteristics of the frequencies of the meridians of the body and for diagnosing whether the meridian is in trouble.

CLM

What is claimed is:

1. A method for monitoring the circulation of blood in a human body having a blood pressure pulse, comprising the steps of: (a) using a pressure transducer to sense the blood pressure pulse in an artery of the body, and produce an electrical pulse representing the blood pressure pulse; (b) using a spectrum analyzer to analyze the frequency spectrum of said electrical pulse in order to display amplitude, frequency, and phase of harmonic components of said electrical pulse; (c) associating the spectral frequencies with selected organs and tissues of the body as predetermined by prior correlation with medical diagnoses and the establishment of a normal spectral pattern; and (d) comparing the harmonic components in the analyzed spectrum to the harmonic components in a normal spectral pattern in order to determine whether or not an organ has abnormal blood circulation.
2. The method as claimed in claim 1, wherein said step (b) includes displaying standard deviation of the measured harmonic components.
3. A method for monitoring the circulation of blood in a human body having a blood pressure pulse, and to evaluate the effectiveness of administering a medical treatment to the human body, comprising the steps of; (a) using a pressure transducer to sense the blood pressure pulse in an artery of the body, and produce an electrical pulse representing the blood pressure pulse; (b) using a spectrum analyzer to analyze the frequency spectrum of said electrical pulse in order to display amplitude, frequency, and phase of harmonic components of said electrical pulse; (c) associating the spectral frequencies with selected organs and tissues of the body as predetermined by prior correlation with medical diagnoses and the establishment of a normal spectral pattern; (d) comparing the harmonic components in the analyzed spectrum to the harmonic components in a normal spectral pattern in order to determine whether or not an organ has abnormal blood circulation; (e) after said comparing step, and upon determining that an organ has abnormal blood circulation, administering treatment to the human body intended to improve the condition of the organ having abnormal blood circulation; (f) repeating steps (a) through (d); and (g) evaluating the effectiveness of administering the treatment by comparing the results of each said comparing step (d).
4. An apparatus for analyzing the blood pressure wave in an artery of the blood circulation of a body, the blood pressure wave having a frequency spectrum comprised of harmonic components, each component having a frequency and a relative phase angle, said apparatus comprising: pressure transducer means adapted to be operatively coupled to a surface of the body for measuring the blood pressure wave of blood flowing through the artery and outputting an electrical pulse representative of the blood pressure wave; and signal analyzer means coupled to and receiving the electrical pulse outputted from said pressure transducer means, said signal analyzer means comprising: means for receiving said outputted electrical pulse from said pressure transducer and analyzing the frequency spectrum of the electrical pulse representing the blood pressure wave in the artery to identify the frequency and relative phase angle of all harmonic components corresponding to respective harmonic frequencies of organs or tissues of the body; and means, coupled to said means for analyzing, for diagnosing an organ or tissue by determining whether or not the organ or tissue is physically abnormal from the amplitude and phase of said analyzed harmonic components, as compared with the amplitude and phase of analyzed harmonic components of a normal, healthy body, at a prescribed relative spectral frequency.
5. An apparatus as set forth in claim 4, wherein: said transducer is adapted to be operatively coupled to the surface of the artery at different locations on the body; and said signal analyzer includes means for diagnosing the condition of blood circulation at different branches of different arteries by analyzing phase and amplitude distribution from said different locations.
6. An apparatus as set forth in claim 4, wherein said signal analyzer comprises harmonics analyzing means for analyzing the harmonics of the heartbeat by Fourier transform techniques.

liver is the first harmonic of the heartbeat, and said harmonics analyzing means evaluates the circulation condition of the liver and its related meridian.

8. An apparatus as set forth in claim 6, where said harmonics analyzing means evaluates the circulation condition of the kidney and its related meridian by analyzing said second harmonic of said heartbeat.

9. An apparatus as set forth in claim 6, wherein said harmonic for the spleen is the third harmonic of said heartbeat, and said harmonics analyzing means evaluates the circulation condition of the spleen and its related meridian.

10. An apparatus as set forth in claim 5, wherein said harmonic for the lung is the fourth harmonic of said heartbeat, and said harmonics analyzing means evaluates the circulation condition of the lung and its related meridian.

11. A method for developing a new medical treatment of a human body, comprising the steps of: (a) employing a pressure transducer to sense, in a human body having a blood pressure pulse, the blood pressure pulse in an artery of the body, and produce an electrical pulse representing the blood pressure pulse; (b) using a spectrum analyzer to analyze the frequency spectrum of said electrical pulse in order to display amplitude, frequency, and phase of harmonic components of said electrical pulse; (c) associating the spectral frequencies with selected organs and tissues of the body as predetermined by prior correlation with medical diagnoses and the establishment of a normal spectral pattern; (d) comparing the harmonic components in the analyzed spectrum to the harmonic components in a normal spectral pattern in order to determine whether or not an organ has abnormal blood circulation; (e) after said comparing step, and upon determining that an organ has abnormal blood circulation, administering treatment to the human body intended to improve the condition of the organ having abnormal blood circulation; (f) repeating steps (a) through (d); (g) evaluating the effectiveness of administering the treatment by comparing the results of each said comparing step (d); (h) repeating steps (a) through (g) until the amount and rate of the effectiveness of the treatment have stabilized; (i) altering an aspect of the treatment; (j) repeating steps (a) through (h); and (k) evaluating the results of each step (h) to determine the best treatment for improving the condition of the organ having abnormal blood circulation.

12. The method as claimed in claim 11, wherein: said administering step (e) comprises administering a drug into the human body; said altering step (i) comprises altering the formula of the drug being administered; and said evaluating step (k) is performed to determine the formula for the drug that resulted in the best amount and rate of effectiveness of the drug treatment.

13. A method for monitoring the circulation of blood in a human body having a blood pressure pulse, comprising the steps of: (a) monitoring the circulation of blood by employing a pressure transducer to sense the blood pressure pulse in an artery of the body, and produce an electrical pulse representing the blood pressure pulse; (b) using a spectrum analyzer to analyze the frequency spectrum of said electrical pulse in order to display amplitude, frequency, and phase of harmonic components of said electrical pulse; (c) associating the spectral frequencies with selected organs and tissues of the body as predetermined by prior correlation with medical diagnoses and the establishment of a normal spectral pattern; and (d) comparing the harmonic components in the analyzed spectrum to the harmonic components in a normal spectral pattern in order to determine whether or not an organ is receiving insufficient oxygen supply.

14. The method as claimed in claim 13, wherein said step (b) includes displaying standard deviation of the measured harmonic components.

15. A method for monitoring the circulation of blood in a human body having a blood pressure pulse, and to evaluate the effectiveness of administering a medical treatment to the human body, comprising the steps of: (a) monitoring the circulation of blood by employing a pressure transducer to sense the blood pressure pulse in an artery of the body, and produce an electrical pulse representing the blood pressure pulse; (b) using a spectrum analyzer to analyze the frequency spectrum of said electrical pulse in order to display amplitude, frequency, and phase of harmonic components of said electrical pulse; (c) associating the spectral frequencies with selected organs and tissues of the body as predetermined by prior correlation with medical diagnoses and the establishment of a normal spectral pattern; (d) comparing the harmonic components in the analyzed spectrum to the harmonic components in a normal spectral pattern in order to determine

after said comparing step, and upon determining that an organ has abnormal blood circulation, administering treatment to the human body intended to improve the condition of the organ having abnormal blood circulation; (f) repeating steps (a) through (d); and (g) evaluating the effectiveness of administering the treatment by comparing the results of each said comparing step (d).

L1 ANSWER 11 OF 12 USPTAFULL on STN

82:4308 Electric safety socket with internal locking means.

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US 4312554 19820126

APPLICATION: US 1980-116715 19800130 (6)

DOCUMENT TYPE: Utility; Granted.

AB An electric socket with locking means inside; when the plug is inserted into it, the electrical source will be connected, and at the same time, the plug will be locked. To disconnect the plug, a button must first be pushed to release the locking means and disconnect the electrical source. This construction will be very safe because there is no way to contact the electrical source from the socket either with the plug inserted or without the plug inserted. When it is connected, accidental disengagement of the plug and disconnection of the electrical source will not occur.

CLM What is claimed is:

1. An electric safety socket with internal locking means, comprising an insulated casing (1), an insulated laterally movable member (3) having a pair of spaced bars (33) for insertion into the contact blades of a plug in a locked position by means of a spring in order that a pair of contact members (4) arranged on said contact bars (33) can be brought into engagement with said contact blades when said plug is inserted into the socket, a pair of spring retainers (2) for retention of said laterally movable member (3) in unlocked position, and a pair of actuators (5) for movement of said laterally movable member.

2. A safety socket with internal locking means as claimed in claim 1, wherein said contact members (4) are plates having an enlarged area for engagement with said contact blades of the plug.

3. A safety socket with internal locking means as claimed in claim 1, wherein said actuators (5) have an elongated extension (511) formed with the cross section of a trapezoid at the intermediate portion thereof.

4. A safety socket with internal locking means as claimed in claim 1, wherein said bars (33) are inserted into holes in the contact blades of the plug.

5. A safety socket with internal locking means as claimed in claim 1, wherein said contact members (4) are removably mounted on said bars (33) by thread means.

L1 ANSWER 12 OF 12 USPTAFULL on STN

80:45353 Method and apparatus for detecting biological particles by fluorescent stain.

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US 4222743 19800916

APPLICATION: US 1978-926342 19780720 (5)

DOCUMENT TYPE: Utility; Granted.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A metal-covered substrate is first adsorbed with a layer of known biological particles. This active substrate is then put into a solution containing an unknown amount of second biological particles that will bind the first kind of particles which are randomly distributed in the layer. A third biological particle bound to a fluorescent dye is applied to this substrate which is then placed in an apparatus having a light source inducing fluorescent emission, and a photon counting system to measure the amount of fluorescent photon. The metal-covered substrate reflects the exciting photon toward a photon trap so that few exciting photons will go to the photon counting system, while the induced signal is reflected toward the photon counting system and more induced signal will be measured.

CLM What is claimed is:

1. Apparatus for detecting biological particles of a particular species in a fluid comprising a substrate having a metal film surface on which has been deposited an immunologic coating of at least one layer which comprises, a layer of protein or protein bound particle adsorbed on said metal film surface with first biological particles randomly distributed therein, means for applying some third biological particles to the substrate after the substrate has been exposed to a fluid containing

particles and means to direct said exciting particles toward said third biological particles; means for measuring the quantity of induced signal from said third biological particle and means to direct the exciting particle which have effected excitation away from the quantity measuring means.

2. The apparatus of claim 1 wherein: said third biological particle comprises a fluorescent dye bound to a biological particle, said exciting particles are photons, said means to direct said exciting particle comprises means for directing photons, said means for measuring the quantity of said induced signal comprises means to measure the quantity of fluorescent emission, said means to direct said exciting photons away from the quantity measuring means comprises said metal surface and a photon trap.

3. The apparatus of claim 2 including additional means for directing photons onto the metal film surface of said substrate.

4. The apparatus of claim 3, wherein said means for directing photons onto the metal film surface of said substrate comprises an enclosure having first and second end members wherein: a photon source within said enclosure adjacent to said first end member, said second member comprises means attached to a surface of said substrate to let the photons to be directed on it, said enclosure also comprises said photon trap.

5. The apparatus of claim 4 further including means in the enclosure for receiving the fluorescent emission.

6. The apparatus of claim 4, wherein said photon source comprises a laser.

7. The apparatus of claim 2, wherein said photon source comprises a lamp and a wavelength selecting device.

8. The apparatus of claim 5, wherein said means comprise a photon counting system and a wavelength selecting device.

9. The apparatus of claim 7, wherein said wavelength selecting device comprises a monochromator.

10. The apparatus of claim 7, wherein said wavelength selecting device comprises a filter.

11. The apparatus of claim 2, wherein said fluorescent dye comprises a fluorescein derivative.

12. The apparatus of claim 11, wherein said fluorescein derivative is fluorescein isothiocyanate.

13. The apparatus of claim 2, wherein said fluorescein dye comprises a rhodamine derivative.

14. The apparatus of claim 13, wherein said rhodamine derivative is tetra methyl rhodamine isothiocyanate.

15. The apparatus of claim 13, wherein said rhodamine dye derivative is lissamine rhodamine B 200 sulfonyl chloride.

16. The apparatus of claim 2, wherein said fluorescent dye is 1-dimethylaminonaphthalene-5-sulfonyl chloride.

17. Method for detecting biological particles of a particular species in a fluid which comprises the steps of: immersing a substrate having a metal surface into a solution of first biological particle, salt and other kinds of protein for sufficient time to adsorb a monomolecular layer of protein, removing unbound protein by washing said substrate with water, immersing said substrate having said monomolecular layer of said protein thereon into said fluid whereby some of a second biological particle, if present in said fluid, is bound to said first biological particle; means to apply third biological particle over the said substrate having said first biological particle and said second biological particle; removing unbound particles by washing said substrate with water, examining said substrate by directing exciting particles toward said substrate, directing the exciting particles away from the quantity measuring means and directing the resulting induced particles toward said quantity measuring means to determine the quantity of the induced signal from the said substrate.

18. The method of claim 17, wherein said means to apply third biological particle comprises the step of immersing said substrate having said first biological particle and said second biological particle if present

biological particle bind to said substrate, and removing unbound particles by washing said substrate with water.

19. The method of claim 17, wherein said means to apply third biological particle comprises the steps of immersing said substrate having said first biological particle and said second biological particle if present into a solution of fourth biological particle to let it bind to said second biological particle, immersing said substrate having said first biological particle, said second biological particle and said fourth biological particle into a solution of third biological particle to let said third biological particle bind to said fourth biological particle, removing unbound particles by washing said substrate with water.

20. The method of claim 18, wherein: said second biological particle is antibody to said first biological particle, said third biological particle is fluorescent dye conjugated antibody to said second biological particle.

21. The method of claim 18, wherein: said first biological is antibody to said second biological particle, said third biological particle is fluorescent dye conjugated antibody to said second biological particle.

22. The method of claim 18, wherein: said second biological particle is antibody to said first biological particle, said third biological particle is fluorescent dye conjugated antibody to the complement of the complex of said first and second biological particle.

23. The method of claim 21, wherein: said fourth biological particle comprises antibody to said second biological particle, said third biological particle is antibody to said fourth biological particle.

24. The method of claim 18, wherein: said second biological particle is a specific binding molecular to said second biological particle, said third biological particle is fluorescent dye conjugated antibody to said second biological particle.

25. The method of claim 18, wherein: one of said first biological particle and said second biological particle is the antibody of the other particle, said third biological particle is the fluorescent dye conjugated second biological particle.

26. The method of claim 18, wherein: one of said first biological particle and said second biological particle is the specific binding molecular of the other particle, said third biological particle is the fluorescent dye conjugated second biological particle.

27. The method of claim 18, wherein examining step more particularly comprises; turning on the light source, directing the light from the light source to the area on said substrate where said third biological particle has been bound.

28. The method of claim 18, wherein said examining step more particularly comprises directing said exciting particles toward said substrate, arranging the substrate to direct the exciting light toward the photon trap, receiving and integrating induced signal from said substrate and indicating the integral quantity of said induced signal to provide a measure of the concentration of said biological particle in said fluid.

29. The apparatus of claim 8, wherein said wavelength selecting device comprises a monochromator.

30. The apparatus of claim 8, wherein said wavelength selecting device comprises a filter.

31. The method of claim 17, wherein said exciting particle are photons and said induced signal comprises fluorescent emission.

32. The apparatus of claim 4, wherein said first member and said second member are so arranged that said exciting photons that enter the enclosure will hit said metal surface and are reflected toward said photon trap.

33. A fluorometric testing apparatus comprising a highly reflective substrate having a surface portion for receiving a sample, means to produce exciting photons and means to direct said exciting photons toward said sample, means for measuring the quantity of the induced photon from said sample, and means to direct the exciting photons which have effected excitation away from the quantity measuring means.

34. The apparatus of claim 33 wherein said highly reflective substrate comprises metal covered substrate, said means to produce exciting

means to direct exciting photons away from the quantity measuring means comprises said metal covered substrate and a photon trap, said means for measuring the quantity of induced photon comprises a wavelength selecting device and a photon counting system.

35. The apparatus of claim 34 wherein said exciting photon after hitting said substrate will be reflected toward said photon trap.

36. The apparatus of claim 35 wherein: said wavelength selecting device of said means to measure the induced photon comprises two pieces of narrow band interference filters with similar peak wavelength.

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(FILE 'HOME' ENTERED AT 22:54:50 ON 03 FEB 2006)

FILE 'USPATFULL' ENTERED AT 22:55:09 ON 03 FEB 2006
E WANG WEI KUNG/IN

L1 12 S E3

=> s (dengue virus)
2606 DENGUE
97109 VIRUS

L2 1163 (DENGUE VIRUS)
(DENGUE(W)VIRUS)

=> s 12 and (PCR or polymerase chain reaction)
81244 PCR
75596 POLYMERASE
589210 CHAIN
839339 REACTION
51193 POLYMERASE CHAIN REACTION
(POLYMERASE(W)CHAIN(W)REACTION)

L3 727 L2 AND (PCR OR POLYMERASE CHAIN REACTION)

=> s 13 and (primer?/clm or oligonucleotide?/clm)
15253 PRIMER?/CLM
14872 OLIGONUCLEOTIDE?/CLM

L4 178 L3 AND (PRIMER?/CLM OR OLIGONUCLEOTIDE?/CLM)

=> s 14 and ay<2003
3786834 AY<2003
L5 112 L4 AND AY<2003

=> d 15,cbib,ab,clm,1-25

L5 ANSWER 1 OF 112 USPATFULL on STN

2005:314715 Methods of enzymatic discrimination enhancement and surface-bound double-stranded DNA.

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US 6974666 B1 20051213

APPLICATION: US 1995-533582 19951018 (8)

DOCUMENT TYPE: Utility; GRANTED.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Methods for discriminating between fully complementary hybrids and those that differ by one or more base pairs and libraries of unimolecular, double-stranded oligonucleotides on a solid support. In one embodiment, the present invention provides methods of using nuclease treatment to improve the quality of hybridization signals on high density oligonucleotide arrays. In another embodiment, the present invention provides methods of using ligation reactions to improve the quality of hybridization signals on high density oligonucleotide arrays. In yet another embodiment, the present invention provides libraries of unimolecular or intermolecular, double-stranded oligonucleotides on a solid support. These libraries are useful in pharmaceutical discovery for the screening of numerous biological samples for specific interactions between the double-stranded oligonucleotides, and peptides, proteins, drugs and RNA. In a related aspect, the present invention provides libraries of conformationally restricted probes on a solid support. The probes are restricted in their movement and flexibility using double-stranded oligonucleotides as scaffolding. The probes are also useful in various screening procedures associated with drug discovery and diagnosis. The present invention further provides methods for the preparation and screening of the above libraries.

CLM What is claimed is:

1. A method for analyzing a target nucleic acid, said method comprising:

synthesized and positionally distinguishable **oligonucleotides** each of which is complementary to a defined subsequence of preselected length; and (ii) a target nucleic acid; thereby forming target-**oligonucleotide** hybrid complexes of complementary subsequences of known sequence; (b) contacting said target-**oligonucleotide** hybrid complexes with a nuclease; thereby removing target-**oligonucleotide** complexes that are not perfectly complementary; and (c) determining which of said **oligonucleotides** have specifically interacted with subsequences in said target nucleic acid as an indication of a subsequence that is complementary to a subsequence of said target nucleic acid.

2. The method as recited in claim 1 wherein said array of **oligonucleotides** recognizes substantially all possible subsequences of preselected length found in said target nucleic acid.

3. The method as recited in claim 1 wherein each **oligonucleotide** is of a length between about 6 and 20 bases.

4. The method as recited in claim 1 wherein each **oligonucleotide** is of a length between about 8 and 15 bases.

5. The method as recited in claim 1 wherein said array of **oligonucleotides** comprises about 1,000 different **oligonucleotides**.

6. The method as recited in claim 1 wherein said array of **oligonucleotides** comprises about 3,000 different **oligonucleotides**.

7. The method as recited in claim 1 wherein said array of **oligonucleotides** comprises about 10' different **oligonucleotides**.

8. The method as recited in claim 1 wherein said array of **oligonucleotides** comprises about 105 different **oligonucleotides**.

9. The method as recited in claim 1 wherein said array of **oligonucleotides** comprises about 10 different **oligonucleotides**.

10. The method as recited in claim 1 wherein said target nucleic acid is ribonucleic acid (RNA).

11. The method as recited in claim 10 wherein said nuclease is an RNA nuclease.

12. The method as recited in claim 11 wherein said RNA nuclease is RNase A.

13. The method as recited in claim 1 wherein said target nucleic acid is deoxyribonucleic acid (DNA).

14. The method as recited in claim 13 wherein said nuclease is a DNA nuclease.

15. The method as recited in claim 14 wherein said DNA nuclease is S1 nuclease.

16. The method as recited in claim 14 wherein said DNA nuclease is Mung Bean nuclease.

17. A method for analyzing an unlabeled target **oligonucleotide**, said method comprising: (a) contacting an unlabeled target **oligonucleotide** with a library of labeled **oligonucleotide** probes, each of said **oligonucleotide** probes having a known sequence and being attached to a solid support at a known position, to hybridize said target **oligonucleotide** to at least one member of said library of probes, thereby forming a hybridized library of target-probe complexes; (b) contacting said hybridized library with a nuclease capable of cleaving double-stranded **oligonucleotides**, thereby preferentially releasing from said hybridized library a portion of said labeled **oligonucleotide** probes or fragments thereof from target-probe complexes that are not perfectly complementary; and (c) identifying said positions of said hybridized library from which labeled probes or fragments thereof have been removed or positions at which labeled proteins remain to determine a subsequence that is complementary to a subsequence of said target **oligonucleotide**.

18. A method for analyzing a target nucleic acid, said method comprising: (a) combining: (i) a substrate comprising an array of chemically synthesized and positionally distinguishable **oligonucleotides** each of which is complementary to a defined subsequence of preselected length; and (ii) a target nucleic acid; thereby forming target-**oligonucleotide** hybrid complexes of complementary subsequences of known sequence and potentially

contacting said target-**oligonucleotide** hybrid complexes with a nuclease under conditions such that said nuclease preferentially cleaves target-**oligonucleotide** hybrid complexes that are not perfectly complementary, including those target-**oligonucleotide** complexes that have only a single mismatch, as compared to perfectly complementary complexes; and (c) determining which of said **oligonucleotides** have specifically interacted with subsequences in said target nucleic acid as an indication of a subsequence that is complementary to a subsequence of said target nucleic acid.

19. The method of claim 18, further comprising washing said array to remove fragments generated by said nuclease.

20. The method of claim 18, wherein said nuclease is a DNA nuclease.

21. The method of claim 20, wherein said nuclease is S1 nuclease.

22. The method of claim 18, wherein said target nucleic acid bears a fluorescent label and detecting comprises obtaining a quantitative fluorescence image of said target-**oligonucleotide** complexes.

23. The method of claim 22, wherein the quantitative fluorescence image is obtained with a confocal microscope.

24. The method of claim 17, wherein (a) the hybridized library potentially includes target-probe complexes that have a single mismatch; and (b) the nuclease preferentially cleaves target-**oligonucleotide** complexes that include a single mismatch as compared to target-**oligonucleotide** complexes that are perfectly complementary.

25. The method of claim 24, wherein said nuclease is a DNA nuclease.

26. The method of claim 25, wherein said nuclease is S1 nuclease.

L5 ANSWER 2 OF 112 USPTAFULL on STN

2005:289009 Human genes and gene expression products II.

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Nuvelo, Inc., Sunnyvale, CA, UNITED STATES (U.S. corporation)

US 6964868 B1 20051115

WO 9938972 19990805

APPLICATION: US 1999-297648 19990128 (9)

WO 1999-US1619 19990128 20000310 PCT 371 date

PRIORITY: US 1999-72910P 19980128 (60)

US 1999-75954P 19980224 (60)

US 1999-80114P 19980331 (60)

US 1999-80515P 19980403 (60)

US 1999-80666P 19980403 (60)

US 1999-105234P 19981021 (60)

US 1999-105877P 19981027 (60)

DOCUMENT TYPE: Utility; GRANTED.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention relates to novel human polynucleotides and variants thereof, their encoded polypeptides and variants thereof, to genes corresponding to these polynucleotides and to proteins expressed by the genes. The invention also relates to diagnostic and therapeutic agents employing such novel human polynucleotides, their corresponding genes or gene products, e.g., these genes and proteins, including probes, antisense constructs, and antibodies.

CLM What is claimed is:

1. An isolated polynucleotide comprising at least 50 contiguous

thereof.

2. A vector comprising a polynucleotide of claim 1.

3. A host cell comprising the vector of claim 2.

4. An isolated polynucleotide comprising at least 50 contiguous nucleotides of SEQ ID NO:253 and which hybridizes under stringent conditions to a polynucleotide of a sequence selected from SEQ ID NO:253 and the complement thereof.

5. The polynucleotide of claim 4, wherein hybridization is conducted at least 50° C. and using 0.1X SSC (9 mM saline/0.9 mM sodium citrate).

6. A polynucleotide comprising at least 50 contiguous nucleotides of either strand of a nucleotide sequence of an insert contained in a vector deposited as clone number M00001448D:C09 of A.T.C.C. Deposit Number 207032, wherein the insert is a human cDNA and the clone is obtained from a human cDNA library.

7. An isolated polynucleotide comprising at least 50 contiguous nucleotides of SEQ ID NO:253, said polynucleotide obtained by amplifying a fragment of cDNA using at least one polynucleotide **primer** comprising at least 15 contiguous nucleotides of a nucleotide sequence selected from the group consisting of: SEQ ID NO: 253 and the complement thereof.

8. A vector comprising a polynucleotide of claim 7.

9. A host cell comprising the vector of claim 8.

L5 ANSWER 3 OF 112 USPATFULL on STN

2005:233477 Apparatus and methods for detecting a microbe in a sample.

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Tilles, Jeremiah G., Irvine, CA, UNITED STATES

The Regents of the University of California, Oakland, CA, UNITED STATES
(U.S. corporation)

US 2005202414 A1 20050915

APPLICATION: US 2002-295787 A1 20021115 (10)

PRIORITY: US 2001-335539P 20011115 (60)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Apparatus for detecting one or more microbes in a sample includes a substrate having a plurality of microbe identification sites with nucleic acid probes disposed thereon, each nucleic acid probe having a nucleotide sequences that is complementary to nucleotide sequences of nucleic acids of one or more microbes. Nucleotide sequences for nucleic acid probes and the primers used to generate the probes are disclosed. Methods of detecting a microbe in a sample using nucleic acid probes are also disclosed.

CLM What is claimed is:

1. Apparatus for detecting the presence of a microbe in a sample, comprising: a substrate having a plurality of microbe identification sites, each microbe identification site having a unique address indicative of the position of that microbe identification site on the substrate; and groups of nucleic acid probes disposed at the microbe identification sites, each group of nucleic acid probes being complementary to a target nucleic acid so as to provide a detectable signal at one or more microbe identification sites.

2. The apparatus of claim 1, wherein the microbe identification sites comprise pathogen identification sites.

3. The apparatus of claim 1, wherein the microbe identification sites comprise viral identification sites.

4. The apparatus of claim 1, wherein the microbe identification sites comprise bacterial identification sites.

5. The apparatus of claim 1, wherein the microbe identification sites comprise pathogenic and non-pathogenic identification sites.

6. The apparatus of claim 1, wherein the microbe identification sites comprise cellular and acellular pathogen identification sites.

7. The apparatus of claim 1, wherein the nucleic acid probes comprise nucleotide sequences that are complementary to genetic sequences of viruses or viroids.

8. The apparatus of claim 1, wherein the nucleic acid probes comprise

respiratory viruses.

9. The apparatus of claim 1, wherein the nucleic acid probes comprise nucleotide sequences that are complementary to a nucleotide sequence of a pathogen selected from the group consisting of: adenoviruses, influenza A virus, influenza B virus, influenza C virus, parainfluenza 1, parainfluenza 2, parainfluenza 3, parainfluenza 4, mumps virus, respiratory syncytial virus, enterovirus, rhinovirus, rubella virus, coronavirus, chlamidia pneumonia, and mycoplasma-pneumonia. ..

10. The apparatus of claim 1, wherein the nucleic acid probes are complementary to a sequence of the target nucleic acid that has at least 80% homology among different types of microbes from a microbe family.

11. The apparatus of claim 10, wherein the nucleic acid probes are complementary to a sequence of the target nucleic acid that has at least 90% homology among different types of microbes from a microbe family.

12. The apparatus of claim 10, wherein the nucleic acid probes are complementary to a sequence of the target nucleic acid that has at least 98% homology among different types of microbes from a microbe family.

13. The apparatus of claim 1, wherein the nucleic acid probes comprise between 65 nucleotides and 80 nucleotides.

14. The apparatus of claim 13, wherein the nucleic acid probes comprise at least 70 nucleotides.

15. The apparatus of claim 13, wherein the nucleic acid probes comprise between 70 nucleotides and 75 nucleotides.

16. The apparatus of claim 1, wherein the nucleic acid probes comprise a nucleotide sequence selected from the group consisting of: SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, and SEQ ID NO: 91.

17. The apparatus of claim 1, wherein a group of nucleic acid probes comprises a plurality of nucleic acids, each nucleic acid of a group having an identical nucleotide sequence.

18. The apparatus of claim 1, wherein the nucleic acid probes are printed on the substrate.

19. The apparatus of claim 1, wherein the substrate comprises a glass slide.

20. The apparatus of claim 1, wherein the substrate comprises a polylysine-coated glass slide.

21. The apparatus of claim 1, wherein the substrate comprises at least one pathogen detection region, and the microbe identification sites are located in the at least one pathogen detection region.

22. The apparatus of claim 1, wherein the target nucleic acid comprises a plurality of nucleic acids amplified by a **polymerase chain reaction**.

23. The apparatus of claim 1, wherein the target nucleic acid comprises a label attached thereto.

24. The apparatus of claim 23, wherein the label comprises a fluorescent label.

25. The apparatus of claim 23, wherein the label comprises Cy3.

26. A kit comprising the apparatus of claim 1, and further comprising a

regions of a target nucleic acid of a microbe to form a nucleic acid comprising about 70 to about 75 bases after a **polymerase chain reaction**.

27. The kit of claim 26, comprising at least one pair of nucleic acid **primers** structured to hybridize to a target nucleic acid of a single microbe.

28. The kit of claim 26, comprising at least two pairs of nucleic acid **primers** structured to hybridize to a target nucleic acid of a microbe.

29. The kit of claim 26, wherein at least one of the nucleic acid **primers** comprise a nucleotide sequence selected from a group consisting of: SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, and SEQ ID NO: 113.

30. The kit of claim 26, further comprising a scanner positioned to receive signals from the apparatus and to scan the microbe detection region for a detectable signal.

31. The kit of claim 26, further comprising an analyzer in communication with the scanner to receive data from the scanner.

32. An apparatus for detecting the presence of a pathogen in a sample, comprising a nucleic acid probe disposed on a substrate, and that hybridizes to a target nucleic acid of a pathogen, the nucleic acid probe comprising a nucleotide sequence selected from a group consisting of: SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, and SEQ ID NO: 91.

33. The apparatus of claim 32, comprising a plurality of nucleic acid probes arranged in groups on the substrate, each group of nucleic acid probes comprising a nucleotide sequence selected from the group consisting of: SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, and SEQ ID NO: 91.

34. A method for detecting the presence of a microbe in a sample, comprising: a) identifying a target nucleic acid of a microbe in a sample; b) labeling the target nucleic acid; c) providing a substrate that has different groups of nucleic acid probes at different locations on the substrate; and d) exposing the labeled target nucleic acid to the substrate such that the labeled target nucleic acid will hybridize to nucleic acid probes that have a nucleotide sequence complementary to

35. The method of claim 34, wherein step (a) comprises identifying a target nucleic acid of a microbe suspected of being present in a biological sample.
36. The method of claim 35, wherein the biological sample comprises a biological fluid.
37. The method of claim 36, wherein the biological fluid is selected from a group consisting of blood, serum, mucus, urine, sputum, saliva, cerebral spinal fluid, and perspiration.
38. The method of claim 34, further comprising a step of amplifying the target nucleic acid using at least one pair of nucleic acid **primers** and a **polymerase chain reaction**.
39. The method of claim 34, further comprising a step of amplifying the target nucleic acid using at least two pairs of nucleic acid **primers** and a **polymerase chain reaction**.
40. The method of claim 34, further comprising a step of amplifying the target nucleic acid and labeling the target nucleic acid during the amplification step.
41. The method of claim 34, further comprising a step of detecting the label at specific locations on the substrate where the labeled nucleic acids hybridized to the nucleic acid probes.
42. The method of claim 34, further comprising a step of detecting a fluorescent signal at specific locations on the substrate where the labeled nucleic acids hybridized to the nucleic acid probes.
43. The method of claim 34, wherein step (a) comprises identifying a target nucleic acid of a plurality of microbes suspected of being present in a sample.
44. The method of claim 34, wherein step (a) comprises identifying a target nucleic acid of a virus or viral particle.
45. The method of claim 34, wherein step (a) comprises identifying a target nucleic acid of a pathogenic or non-pathogenic bacteria.
46. The method of claim 34, wherein step (a) comprises identifying a target nucleic acid of a cellular or acellular microbe.

L5 ANSWER 4 OF 112 USPATFULL on STN

2005:144275 Whole cell engineering by mutagenizing a substantial portion of a starting genome combining mutations and optionally repeating.

Short, Jay M, Rancho Santa Fe, CA, UNITED STATES

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APPLICATION: US 2003-398271 A1 20011001 (10)

WO 2001-US31004 20011001

PRIORITY: US 2003-9677584 20000930

US 2003-279702P 20010328 (60)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention relates to the field of cellular and whole organism engineering. Specifically, this invention relates to a cellular transformation, directed evolution, and screening method for creating novel transgenic organisms having desirable properties. Thus in one aspect, this invention relates to a method of generating a transgenic organism, such as a microbe or a plant, having a plurality of traits that are differentially activatable.

CLM What is claimed is:

1. A method for identifying proteins by differential labeling of peptides, the method comprising the following steps: (a) providing a sample comprising a polypeptide; (b) providing a plurality of labeling reagents which differ in molecular mass that can generate differential labeled peptides that do not differ in chromatographic retention properties and do not differ in ionization and detection properties in mass spectrographic analysis, wherein the differences in molecular mass are distinguishable by mass spectrographic analysis; (c) fragmenting the polypeptide into peptide fragments by enzymatic digestion or by non-enzymatic fragmentation; (d) contacting the labeling reagents of step (b) with the peptide fragments of step (c), thereby labeling the peptides with the differential labeling reagents; (e) separating the peptides by chromatography to generate an eluate; (f) feeding the

of each peptide and generating the sequence of each peptide by use of the mass spectrometer; (g) inputting the sequence to a computer program product which compares the inputted sequence to a database of polypeptide sequences to identify the polypeptide from which the sequenced peptide originated.

2. The method of claim 1, wherein the sample of step (a) comprises a cell or a cell extract.

3. The method of claim 1, further comprising providing two or more samples comprising a polypeptide.

4. The method of claim 3, wherein one sample is derived from a wild type cell and one sample is derived from an abnormal or a modified cell.

5. The method of claim 4, wherein the abnormal cell is a cancer cell.

6. The method of claim 1, further comprising purifying or fractionating the polypeptide before the fragmenting of step (c).

7. The method of claim 1, further comprising purifying or fractionating the polypeptide before the labeling of step (d).

8. The method of claim 1, further comprising purifying or fractionating the labeled peptide before the chromatography of step (e).

9. The method of claim 6, claim 8 or claim 8, wherein the purifying or fractionating comprises a method selected from the group consisting of size exclusion chromatography, size exclusion chromatography, HPLC, reverse phase HPLC and affinity purification.

10. The method of claim 1, further comprising contacting the polypeptide with a labeling reagent of step (b) before the fragmenting of step (c).

11. The method of claim 1, wherein the labeling reagent of step (b) comprises the general formulae selected from the group consisting of:

i. $ZAOH$ and $ZBOH$, to esterify peptide C-terminals and/or Glu and Asp side chains; ii. $ZANH_2$ and $ZBNH_2$, to form amide bond with peptide C-terminals and/or Glu and Asp side chains; and iii. $ZACO_2H$ and $ZBCO_2H$, to form amide bond with peptide N-terminals and/or Lys and Arg side chains; wherein Z^A and Z^B independently of one another comprise the general formula $R-Z^1-A^1-Z^2-A^2-Z^3-A^3-Z^4-A^4-$, Z^1 , Z^2 , Z^3 , and Z^4 independently of one another, are selected from the group consisting of nothing, O , $OC(O)$, $OC(S)$, $OC(O)O$, $OC(O)NR$, $OC(S)NR$, $OSiRR^1$, S , $SC(O)$, $SC(S)$, SS , $S(O)$, $S(O_2)$, NR , NRR^1+ , $C(O)$, $C(O)O$, $C(S)$, $C(S)O$, $C(O)S$, $C(O)NR$, $C(S)NR$, $SiRR^1$, $(Si(RR^1)O)_n$, $SnRR^1$, $Sn(RR^1)O$, $BR(OR^1)$, BRR^1 , $B(OR)(OR^1)$, $OBR(OR^1)$, $OBRR^1$, and $OB(OR)(OR^1)$, and R and R^1 is an alkyl group, A^1 , A^2 , A^3 , and A^4 independently of one another, are selected from the group consisting of nothing or $(CRR^1)_n$, wherein R , R^1 , independently from other R and R^1 in Z^1 to Z^4 and independently from other R and R^1 in A^1 to A^4 , are selected from the group consisting of a hydrogen atom, a halogen atom and an alkyl group; n in Z^1 to Z^4 , independent of n in A^1 to A^4 , is an integer having a value selected from the group consisting of 0 to about 51; 0 to about 41; 0 to about 31; 0 to about 21, 0 to about 11 and 0 to about 6.

12. The method of claim 11, wherein the alkyl group is selected from the group consisting of an alkenyl, an alkynyl and an aryl group.

13. The method of claim 11, wherein one or more C--C bonds from $(CRR^1)_n$ are replaced with a double or a triple bond,

14. The method of claim 13, wherein an R or an R^1 group is deleted.

15. The method of claim 13, wherein $(CRR^1)_n$ is selected from the group consisting of an o-arylene, an m-arylene and a p-arylene, wherein each group has none or up to 6 substituents.

16. The method of claim 13, wherein $(CRR^1)_n$ is selected from the group consisting of a carbocyclic, a bicyclic and a tricyclic fragment, wherein the fragment has up to 8 atoms in the cycle with or without a heteroatom selected from the group consisting of an O atom, a N atom and an S atom.

17. The method of claim 1, wherein two or more labeling reagents have the same structure but a different isotope composition.

18. The method of claim 11, wherein Z^A has the same structure as

19. The method of claim 17, wherein the isotope is boron-10 and boron-11.
20. The method of claim 17, wherein the isotope is carbon-12 and carbon-13.
21. The method of claim 17, wherein the isotope is nitrogen-14 and nitrogen-15.
22. The method of claim 17, wherein the isotope is sulfur-32 and sulfur-34.
23. The method of claim 17, wherein, where the isotope with the lower mass is x and the isotope with the higher mass is y, and x and y are integers, x is greater than y.
24. The method of claim 17, wherein x and y are between 1 and about 11, between 1 and about 21, between 1 and about 31, between 1 and about 41, or between 1 and about 51.
25. The method of claim 1, wherein the labeling reagent of step (b) comprises the general formulae selected from the group consisting of:
 - i. $\text{CD}_3(\text{CD}_2)_{\text{nOH/CH}_3(\text{CH}_2)_{\text{nOH}}}$, to esterify peptide C-terminals, where n=0, 1, 2 or y; ii. $\text{CD}_3(\text{CD}_2)_{\text{nNH}_2} \text{CH}_3(\text{CH}_2)_{\text{nNH}_2}$, to form amide bond with peptide C-terminals, where n=0, 1, 2 or y; and
 - iii. $\text{D}(\text{CD}_2)_{\text{nCO}_2\text{H/H}(\text{CH}_2)_{\text{nCO}_2\text{H}}}$, to form amide bond with peptide N-terminals, where n=0, 1, 2 or y; wherein D is a deuterium atom, and y is an integer selected from the group consisting of about 51; about 41; about 31; about 21, about 11; about 6 and between about 5 and 51.
26. The method of claim 1, wherein the labeling reagent of step (b) comprises the general formulae selected from the group consisting of:
 - i. Z^{AOH} and Z^{BOH} to esterify peptide C-terminals; ii. $\text{Z}^{\text{ANH}_2/\text{Z}^{\text{BNH}_2}}$ to form an amide bond with peptide C-terminals; and iii. $\text{Z}^{\text{ACO}_2\text{H}/\text{Z}^{\text{BCO}_2\text{H}}}$ to form an amide bond with peptide N-terminals; wherein Z^{A} and Z^{B} have the general formula $\text{R-Z}^1\text{-A}^1\text{-Z}^2\text{-A}^2\text{-Z}^3\text{-A}^3\text{-Z}^4\text{-A}^4\text{-Z}^1$, Z^2 , Z^3 , and Z^4 , independently of one another, are selected from the group consisting of nothing, 0, OC(O) , OC(S) , OC(O)O , OC(O)NR , OC(S)NR , OSiRR^1 , S, SC(O) , SC(S) , SS, S(O) , $\text{S(O}_2\text{)}$, NR, NRR^1 , C(O) , C(O)O , C(S) , C(S)O , C(O)S , C(O)NR , C(S)NR , SiRR^1 , $(\text{Si}(\text{RR}^1)\text{O})_n$, SnRR^1 , $\text{Sn}(\text{RR}^1)\text{O}$, $\text{BR(OR}^1\text{)}$, BRR^1 , $\text{B(OR)(OR}^1\text{)}$, $\text{OBR(OR}^1\text{)}$, OBRR^1 , and $\text{OB(OR)(OR}^1\text{)}$; A^1 , A^2 , A^3 , and A^4 , independently of one another, are selected from the group consisting of nothing and the general formulae $(\text{CRR}^1)_n$, and, R and R^1 is an alkyl group.
27. The method of claim 26, wherein a single C--C bond in a $(\text{CRR}^1)_n$ group is replaced with a double or a triple bond.
28. The method of claim 27, wherein R and R^1 are absent.
29. The method of claim 27, wherein $(\text{CRR}^1)_n$ comprises a moiety selected from the group consisting of an o-arylene, an m-arylene and ap-arylene, wherein the group has none or up to 6 substituents.
30. The method of claim 27, wherein the group comprises a carbocyclic, a bicyclic, or a tricyclic fragments with up to 8 atoms in the cycle, with or without a heteroatom selected from the group consisting of an O atom, an N atom and an S atom.
31. The method of claim 26, wherein R, R^1 , independently from other R and R^1 in $\text{Z}^1\text{-Z}^4$ and independently from other R and R^1 in $\text{A}^1\text{-A}^4$, are selected from the group consisting of a hydrogen atom, a halogen and an alkyl group.
32. The method of claim 31, wherein the alkyl group is selected from the group consisting of an alkenyl, an alkynyl and an aryl group.
33. The method of claim 26, wherein n in $\text{Z}^1\text{-Z}^4$ is independent of n in $\text{A}^1\text{-A}^4$ and is an integer selected from the group consisting of about 51; about 41; about 31; about 21, about 11 and about 6.
34. The method of claim 26, wherein Z^{A} has the same structure as Z^{B} but Z^{A} further comprises x number of $-\text{CH}_2-$ fragment(s) in one or more $\text{A}^1\text{-A}^4$ fragments, wherein x is an

35. The method of claim 26, wherein Z^A has the same structure as Z^B but Z^A further comprises x number of --CF₂-- fragment(s) in one or more A¹-A⁴ fragments, wherein x is an integer.
36. The method of claim 26, wherein Z^A comprises x number of protons and Z^B comprises y number of halogens in the place of protons, wherein x and y are integers.
37. The method of claim 26, wherein Z^A contains x number of protons and Z^B contains y number of halogens, and there are x-y number of protons remaining in one or more A¹-A⁴ fragments, wherein x and y are integers
38. The method of claim 26, wherein Z^A further comprises x number of --O-- fragment(s) in one or more A¹-A⁴ fragments, wherein x is an integer.
39. The method of claim 26, wherein Z^A further comprises x number of --S-- fragment(s) in one or more A¹-A⁴ fragments, wherein x is an integer.
40. The method of claim 26, wherein Z^A further comprises x number of --O-- fragment(s) and Z^B further comprises y number of --S-- fragment(s) in the place of --O-- fragment(s), wherein x and y are integers.
41. The method of claim 26, wherein Z^A further comprises x-y number of --O-- fragment(s) in one or more A¹-A⁴ fragments, wherein x and y are integers.
42. The method of claim 37, claim 40 or claim 41, wherein x and y are integers selected from the group consisting of between 1 about 51; between 1 about 41; between 1 about 31; between 1 about 21, between 1 about 11 and between 1 about 6, wherein x is greater than y.
43. The method of claim 1, wherein the labeling reagent of step (b) comprises the general formulae selected from the group consisting of:
i. CH₃(CH₂)_{nOH/CH₃}(CH₂)_n+mOH, to esterify peptide C-terminals, where n=0, 1, 2, . . . , y; m=1, 2, . . . y; ii. CH₃(CH₂)_n NH₂/CH₃(CH₂)_n+mNH₂, to form amide bond with peptide C-terminals, where n=0, 1, 2, . . . , y; m=1, 2, . . . , y; and, iii. H(CH₂)_{nCO₂H/H}(CH₂)_{sub}.n+mCO₂H, to form amide bond with peptide N-terminals, where n=0, 1, 2, . . . , y; m=1, 2, . . . , y; wherein n, m and y are integers.
44. The method of claim 43, wherein n, m and y are integers selected from the group consisting of about 51; about 41; about 31; about 21, about 11; about 6 and between about 5 and 51.
45. The method of claim 1, wherein the separating of step (e) comprises a liquid chromatography system.
46. The method of claim 1, wherein the liquid chromatography system comprises a multidimensional liquid chromatography.
47. The method of claim 1, wherein the mass spectrometer comprises a tandem mass spectrometry device.
48. The method of claim 1, further comprising quantifying the amount of each polypeptide.
49. The method of claim 1, further comprising quantifying the amount of each peptide.
50. A method for defining the expressed proteins associated with a given cellular state, the method comprising the following steps: (a) providing a sample comprising a cell in the desired cellular state; (b) providing a plurality of labeling reagents which differ in molecular mass but do not differ in chromatographic retention properties and do not differ in ionization and detection properties in mass spectrographic analysis, wherein the differences in molecular mass are distinguishable by mass spectrographic analysis; (c) fragmenting polypeptides derived from the cell into peptide fragments by enzymatic digestion or by non-enzymatic fragmentation; (d) contacting the labeling reagents of step (b) with the peptide fragments of step (c), thereby labeling the peptides with the differential labeling reagents; (e) separating the peptides by chromatography to generate an eluate; (f) feeding the eluate of step (e) into a mass spectrometer and quantifying the amount of each peptide and generating the sequence of each peptide by use of the mass spectrometer; (g) inputting the sequence to a computer program

polypeptide sequences to identify the polypeptide from which the sequenced peptide originated, thereby defining the expressed proteins associated with the cellular state.

51. A method for quantifying changes in protein expression between at least two cellular states, the method comprising the following steps: state; (b) providing a plurality of labeling reagents which differ in molecular mass but do not differ in chromatographic retention properties and do not differ in ionization and detection properties in mass spectrographic analysis, wherein the differences in molecular mass are distinguishable by mass spectrographic analysis; (c) fragmenting polypeptides derived from the cells into peptide fragments by enzymatic digestion or by non-enzymatic fragmentation; (d) contacting the labeling reagents of step (b) with the peptide fragments of step (c), thereby labeling the peptides with the differential labeling reagents, wherein the labels used in one same are different from the labels used in other samples; (e) separating the peptides by chromatography to generate an eluate; (f) feeding the eluate of step (e) into a mass spectrometer and quantifying the amount of each peptide and generating the sequence of each peptide by use of the mass spectrometer; (g) inputting the sequence to a computer program product which identifies from which sample each peptide was derived, compares the inputted sequence to a database of polypeptide sequences to identify the polypeptide from which the sequenced peptide originated, and compares the amount of each polypeptide in each sample, thereby quantifying changes in protein expression between at least two cellular states.

52. A method for identifying proteins by differential labeling of peptides, the method comprising the following steps: (a) providing a sample comprising a polypeptide; (b) providing a plurality of labeling reagents which differ in molecular mass but do not differ in chromatographic retention properties and do not differ in ionization and detection properties in mass spectrographic analysis, wherein the differences in molecular mass are distinguishable by mass spectrographic analysis; (c) fragmenting the polypeptide into peptide fragments by enzymatic digestion or by non-enzymatic fragmentation; (d) contacting the labeling reagents of step (b) with the peptide fragments of step (c), thereby labeling the peptides with the differential labeling reagents; (e) separating the peptides by multidimensional liquid chromatography to generate an eluate; (f) feeding the eluate of step (e) into a tandem mass spectrometer and quantifying the amount of each peptide and generating the sequence of each peptide by use of the mass spectrometer; (g) inputting the sequence to a computer program product which compares the inputted sequence to a database of polypeptide sequences to identify the polypeptide from which the sequenced peptide originated.

53. A chimeric labeling reagent comprising (a) a first domain comprising a biotin; and (b) a second domain comprising a reactive group capable of covalently binding to an amino acid, wherein the chimeric labeling reagent comprises at least one isotope.

54. The chimeric labeling reagent of claim 53, wherein the isotope is in the first domain.

55. The chimeric labeling reagent of claim 54, wherein the isotope is in the biotin.

56. The chimeric labeling reagent of claim 53, wherein the isotope is in the second domain.

57. The chimeric labeling reagent of claim 53, wherein the isotope is selected from the group consisting of a deuterium isotope, a boron-10 or boron-11 isotope, a carbon-12 or a carbon-13 isotope, a nitrogen-14 or a nitrogen-15 isotope and a sulfur-32 or a sulfur-34 isotope.

58. The chimeric labeling reagent of claim 53 comprising two or more isotopes.

59. The chimeric labeling reagent of claim 53, wherein the reactive group capable of covalently binding to an amino acid is selected from the group consisting of a succimide group, an isothiocyanate group and an isocyanate group.

60. The chimeric labeling reagent of claim 53, wherein the reactive group capable of covalently binding to an amino acid binds to a lysine or a cysteine.

61. The chimeric labeling reagent of claim 53, further comprising a linker moiety linking the biotin group and the reactive group.

62. The chimeric labeling reagent of claim 53; wherein the linker moiety

63. The chimeric labeling reagent of claim 53, wherein the linker is a cleavable moiety.
64. The chimeric labeling reagent of claim 53, wherein the linker can be cleaved by enzymatic digest.
65. The chimeric labeling reagent of claim 53, wherein the linker can be cleaved by reduction.
66. A method of comparing relative protein concentrations in a sample comprising (a) providing a plurality of differential small molecule tags, wherein the small molecule tags are structurally identical but differ in their isotope composition, and the small molecules comprise reactive groups that covalently bind to cysteine or lysine residues or both; (b) providing at least two samples comprising polypeptides; (c) attaching covalently the differential small molecule tags to amino acids of the polypeptides; (d) determining the protein concentrations of each sample in a tandem mass spectrometer; and, (d) comparing relative protein concentrations of each sample.
67. The method of claim 66, wherein the sample comprises a complete or a fractionated cellular sample.
68. The method of claim 66, wherein differential small molecule tags comprise a chimeric labeling reagent comprising (a) a first domain comprising a biotin; and, (b) a second domain comprising a reactive group capable of covalently binding to an amino acid, wherein the chimeric labeling reagent comprises at least one isotope.
69. The method of claim 68, wherein the isotope is selected from the group consisting of a deuterium isotope, a boron-10 or boron-11 isotope, a carbon-12 or a carbon-13 isotope, a nitrogen-14 or a nitrogen-15 isotope and a sulfur-32 or a sulfur-34 isotope.
70. The method of claim 68, wherein the chimeric labeling reagent comprises two or more isotopes.
71. The method of claim 68, wherein the reactive group capable of covalently binding to an amino acid is selected from the group consisting of a succinimide group, an isothiocyanate group and an isocyanate group.
72. A method of comparing relative protein concentrations in a sample comprising (a) providing a plurality of differential small molecule tags, wherein the differential small molecule tags comprise a chimeric labeling reagent comprising (i) a first domain comprising a biotin; and, (ii) a second domain comprising a reactive group capable of covalently binding to an amino acid, wherein the chimeric labeling reagent comprises at least one isotope; (b) providing at least two samples comprising polypeptides; (c) attaching covalently the differential small molecule tags to amino acids of the polypeptides; (d) isolating the tagged polypeptides on a biotin-binding column by binding tagged polypeptides to the column, washing non-bound materials off the column, and eluting tagged polypeptides off the column; (e) determining the protein concentrations of each sample in a tandem mass spectrometer; and, (f) comparing relative protein concentrations of each sample.
73. A method of producing an improved organism having a desirable trait comprising: a) obtaining an initial population of organisms, b) generating a set of mutagenized organisms, such that when all the genetic mutations in the set of mutagenized organisms are taken as a whole, there is represented a set of substantial genetic mutations, and c) detecting the presence of said improved organism.
74. The method of claim 73, wherein the set of substantial genetic mutations in step b) is comprised of a knocking out of at least 15 different genes.
75. The method of claim 73, wherein the set of substantial genetic mutations in step b) is comprised of a knocking out of at least 50 different genes.
76. The method of claim 73, wherein the set of substantial genetic mutations in step b) is comprised of a knocking out of at least 100 different genes.
77. The method of claim 73, wherein the set of substantial genetic mutations in step b) is comprised of an introduction of at least 15 different genes.
78. The method of claim 73, wherein the set of substantial genetic

different genes.

79. The method of claim 73, wherein the set of substantial genetic mutations in step b) is comprised of an introduction of at least 100 different genes.

80. The method of claim 73, wherein the set of substantial genetic mutations in step b) is comprised of an alteration in the expression of at least 15 different genes.

81. The method of claim 73, wherein the set of substantial genetic mutations in step b) is comprised of an alteration in the expression of at least 50 different genes.

82. The method of claim 73, wherein the set of substantial genetic mutations in step b) is comprised of an alteration in the expression of at least 100 different genes.

83. A method of producing an improved organism having a desirable trait comprising: a) obtaining an initial population of organisms, b) generating a set of mutagenized organisms each having at least one genetic mutation, such that when all the genetic mutations in the set of mutagenized organisms are taken as a whole, there is represented a set of substantial genetic mutations c) detecting the manifestation of at least two genetic mutations, d) introducing at least two detected genetic mutations into one organism, and e) optionally repeating any of steps a), b), c), and d).

84. The method of claim 83, wherein step d) is comprised of a knocking out of at least 15 different genes in one organism.

85. The method of claim 83, wherein step d) is comprised of a knocking out of at least 50 different genes in one organism.

86. The method of claim 83, wherein step d) is comprised of a knocking out of at least 100 different genes in one organism.

87. The method of claim 83, wherein step d) is comprised of an introduction of at least 15 different genes into one organism.

88. The method of claim 83, wherein step d) is comprised of an introduction of at least 50 different genes into one organism.

89. The method of claim 83, wherein step d) is comprised of an introduction of at least 100 different genes into one organism.

90. The method of claim 83, wherein step d) is comprised of an alteration in the expression of at least 15 different genes in one organism.

91. The method of claim 83, wherein step d) is comprised of an alteration in the expression of at least 50 different genes in one organism.

92. The method of claim 83, wherein step d) is comprised of an alteration in the expression of at least 100 different genes in one organism.

93. A method for identifying a gene that alters a trait of an organism, comprising: a) obtaining an initial population of organisms, b) generating a set of mutagenized organisms, such that when all the genetic mutations in the set of mutagenized organisms are taken as a whole, there is represented a set of substantial genetic mutations, and c) detecting the presence an organism having said altered trait, and d) determining the nucleotide sequence of a gene that has been mutagenized in the organism having the altered trait.

94. A method for producing an organism with an improved trait, comprising: a) functionally knocking out an endogenous gene in a substantially clonal population of organisms; b) transferring a library of altered genes into the substantially clonal population of organisms, wherein each altered gene differs from the endogenous gene at only one codon; c) detecting a mutagenized organism having an improved trait; and d) determining the nucleotide sequence of a gene that has been transferred into the detected organism.

95. A method of introducing differentially activatable stacked traits into a transgenic cell or organism, which method is comprised of the following steps: a) obtaining an initial cell or organism; b) introducing into the working cell or organism a plurality of traits (stacked traits), including selectively and differentially activatable traits, whereby serviceable traits for this purpose include traits

the information obtained from steps a) and b), and d) optionally repeating any number or all of the steps of a), b), c), and d);

96. The method of claim 95, wherein step a) also includes holistic monitoring of the strain or organism whereby holistic monitoring can include the detection and/or measurement of all detectable functions and physical parameters (such as but not limited to morphology, behavior, growth, responsiveness to stimuli [e.g., antibiotics, different environment, etc.], and profiles of all detectable molecules, including molecules that are chemically at least in part a nucleic acids, proteins, carbohydrates, proteoglycans, glycoproteins, or lipids)

97. The method of claim 95, wherein step d) also includes holistic monitoring of the strain or organism whereby holistic monitoring can include the detection and/or measurement of all detectable functions and physical parameters (such as but not limited to morphology, behavior, growth, responsiveness to stimuli [e.g., antibiotics, different environment, etc.], and profiles of all detectable molecules, including molecules that are chemically at least in part a nucleic acids, proteins, carbohydrates, proteoglycans, glycoproteins, or lipids)

98. The method of claim 95, wherein step a) and d) include holistic monitoring of the strain or organism whereby holistic monitoring can include the detection and/or measurement of all detectable functions and physical parameters (such as but not limited to morphology, behavior, growth, responsiveness to stimuli [e.g., antibiotics, different environment, etc.], and profiles of all detectable molecules, including molecules that are chemically at least in part a nucleic acids, proteins, carbohydrates, proteoglycans, glycoproteins, or lipids)

99. The method of claim 95, wherein step b) includes the introduction of at least 15 stacked traits

100. The method of claim 95, wherein step b) includes the introduction of at least 50 stacked traits

101. The method of claim 95, wherein step b) includes the introduction of at least 100 stacked traits

102. The method of claim 96, wherein step a) includes screening cellular characteristics by utilizing one or any combination of the following methods: a) genomics; b) transcriptome characterization or RNA profiling; c) proteomics; d) metabolomics or the analysis of metabolites; e) lipidomics or lipid profiling.

103. A method of claim 102, wherein proteomics specifically includes the use of amino acid reactive tags

104. A method of claim 97, wherein step d) includes screening cellular characteristics by utilizing one or any combination of the following methods: f) genomics; g) transcriptome characterization or RNA profiling; h) proteomics; i) metabolomics or the analysis of metabolites; j) lipidomics or lipid profiling.

105. A method of claim 104, wherein proteomics specifically includes the use of amino acid reactive tags

106. A method of claim 98, wherein steps a) and d) include screening cellular characteristics by utilizing one or any combination of the following methods: k) genomics; l) transcriptome characterization or RNA profiling; m) proteomics; n) metabolomics or the analysis of metabolites; o) lipidomics or lipid profiling. P)

107. A method of claim 106, wherein proteomics specifically includes the use of amino acid reactive tags

108. A method of claim 73, wherein step c) includes screening cellular characteristics by utilizing one or any combination of the following methods: q) genomics; r) transcriptome characterization or RNA profiling; s) proteomics; t) metabolomics or the analysis of metabolites; u) lipidomics or lipid profiling.

109. A method of claim 108, wherein proteomics specifically includes the use of amino acid reactive tags

110. A method of claim 93, wherein step c) includes screening cellular characteristics by utilizing one or any combination of the following methods: v) genomics; w) transcriptome characterization or RNA profiling; x) proteomics; y) metabolomics or the analysis of metabolites; z) lipidomics or lipid profiling.

111. A method of claim 110, wherein proteomics specifically includes the

112. A method of claim 94, wherein step c) includes screening cellular characteristics by utilizing one or any combination of the following methods: aa) genomics; bb) transcriptome characterization or RNA profiling; cc) proteomics; dd) metabolomics or the analysis of metabolites; ee) lipidomics or lipid profiling.

113. A method of claim 112, wherein proteomics specifically includes the use of amino acid reactive tags

114. A method for whole cell engineering of new or modified phenotypes by using real-time metabolic flux analysis, the method comprising the following steps: (a) making a modified cell by modifying the genetic composition of a cell; (b) culturing the modified cell to generate a plurality of modified cells; (c) measuring at least one metabolic parameter of the cell by monitoring the cell culture of step (b) in real time; and, (d) analyzing the data of step (c) to determine if the measured parameter differs from a comparable measurement in an unmodified cell under similar conditions, thereby identifying an engineered phenotype in the cell using real-time metabolic flux analysis.

115. The method of claim 114, wherein the genetic composition of the cell is modified by a method comprising addition of a nucleic acid to the cell.

116. The method of claim 115, wherein the nucleic acid comprises a nucleic acid heterologous to the cell.

117. The method of claim 115, wherein the nucleic acid comprises a nucleic acid homologous to the cell.

118. The method of claim 117, wherein the homologous nucleic acid comprises a modified homologous nucleic acid.

119. The method of claim 118, wherein the homologous nucleic acid comprises a modified homologous gene.

120. The method of claim 114, wherein the genetic composition of the cell is modified by a method comprising deletion of a sequence or modification of a sequence in the cell.

121. The method of claim 114, wherein the genetic composition of the cell is modified by a method comprising modifying or knocking out the expression of a gene.

122. The method of claim 114, further comprising selecting a cell comprising a newly engineered phenotype.

123. The method of claim 122, further comprising culturing the selected cell, thereby generating a new cell strain comprising a newly engineered phenotype.

124. The method of claim 122, wherein the newly engineered phenotype is selected from the group consisting of an increased or decreased expression or amount of a polypeptide, an increased or decreased amount of an mRNA transcript, an increased or decreased expression of a gene, an increased or decreased resistance or sensitivity to a toxin, an increased or decreased resistance use or production of a metabolite, an increased or decreased uptake of a compound by the cell, an increased or decreased rate of metabolism, and an increased or decreased growth rate.

125. The method of claim 114, further comprising isolating a cell comprising a newly engineered phenotype.

126. The method of claim 114, wherein the newly engineered phenotype is a stable phenotype.

127. The method of claim 126, wherein modifying the genetic composition of a cell comprises insertion of a construct into the cell, wherein construct comprises a nucleic acid operably linked to a constitutively active promoter.

128. The method of claim 114, wherein the newly engineered phenotype is an inducible phenotype.

129. The method of claim 128, wherein modifying the genetic composition of a cell comprises insertion of a construct into the cell, wherein construct comprises a nucleic acid operably linked to an inducible promoter.

130. The method of claim 115, wherein nucleic acid added to the cell in

131. The method of claim 115, wherein nucleic acid added to the cell in step (a) propagates as an episome in the cell.

132. The method of claim 115, wherein nucleic acid added to the cell in step (a) encodes a polypeptide.

133. The method of claim 132, wherein the polypeptide comprises a modified homologous polypeptide.

134. The method of claim 132, wherein the polypeptide comprises a heterologous polypeptide.

135. The method of claim 115, wherein the nucleic acid added to the cell in step (a) encodes a transcript comprising a sequence that is antisense to a homologous transcript.

136. The method of claim 114, wherein modifying the genetic composition of the cell in step (a) comprises increasing or decreasing the expression of an mRNA transcript.

137. The method of claim 114, wherein modifying the genetic composition of the cell in step (a) comprises increasing or decreasing the expression of a polypeptide.

138. The method of claim 114, wherein modifying the homologous gene in step (a) comprises knocking out expression of the homologous gene.

139. The method of claim 114, wherein modifying the homologous gene in step (a) comprises increasing the expression of the homologous gene.

140. The method of claim 114, wherein the heterologous gene in step (a) comprises a sequence-modified homologous gene, wherein the sequence modification is made by a method comprising the following steps: (a) providing a template polynucleotide, wherein the template polynucleotide comprises a homologous gene of the cell; (b) providing a plurality of **oligonucleotides**, wherein each **oligonucleotide** comprises a sequence homologous to the template polynucleotide, thereby targeting a specific sequence of the template polynucleotide, and a sequence that is a variant of the homologous gene; (c) generating progeny polynucleotides comprising non-stochastic sequence variations by replicating the template polynucleotide of step (a) with the **oligonucleotides** of step (b), thereby generating polynucleotides comprising homologous gene sequence variations.

141. The method of claim 114, wherein the heterologous gene in step (a) comprises a sequence-modified homologous gene, wherein the sequence modification is made by a method comprising the following steps: (a) providing a template polynucleotide, wherein the template polynucleotide comprises sequence encoding a homologous gene; (b) providing a plurality of building block polynucleotides, wherein the building block polynucleotides are designed to cross-over reassemble with the template polynucleotide at a predetermined sequence, and a building block polynucleotide comprises a sequence that is a variant of the homologous gene and a sequence homologous to the template polynucleotide flanking the variant sequence; (c) combining a building block polynucleotide with a template polynucleotide such that the building block polynucleotide cross-over reassembles with the template polynucleotide to generate polynucleotides comprising homologous gene sequence variations.

142. The method of claim 114, wherein the cell is a prokaryotic cell.

143. The method of claim 142, wherein the prokaryotic cell is a bacterial cell.

144. The method of claim 114, wherein the cell is selected from the group consisting of a fungal cell, a yeast cell, a plant cell and an insect cell.

145. The method of claim 114, wherein the cell is a eukaryotic cell.

146. The method of claim 145, wherein the cell is a mammalian cell.

147. The method of claim 146, wherein the mammalian cell is a human cell.

148. The method of claim 114, wherein the measured metabolic parameter comprises rate of cell growth.

149. The method of claim 148, wherein the rate of cell growth is measured by a change in optical density of the culture.

150. The method of claim 114, wherein the measured metabolic parameter comprises a change in the expression of a polypeptide.

151. The method of claim 150, wherein the change in the expression of the polypeptide is measured by a method selected from the group consisting of a one-dimensional gel electrophoresis, a two-dimensional gel electrophoresis, a tandem mass spectrometry, an RIA, an ELISA, an immunoprecipitation and a Western blot.

152. The method of claim 114, wherein the measured metabolic parameter comprises a change in expression of at least one transcript, or, the expression of a transcript of a newly introduced gene.

153. The method of claim 152, wherein the change in expression of the transcript is measured by a method selected from the group consisting of a hybridization, a quantitative amplification and a Northern blot.

154. The method of claim 153, wherein transcript expression is measured by hybridization of a sample comprising transcripts of a cell or nucleic acid representative of or complementary to transcripts of a cell by hybridization to immobilized nucleic acids on an array.

155. The method of claim 114, wherein the measured metabolic parameter comprises an increase or a decrease in a secondary metabolite.

156. The method of claim 155, wherein secondary metabolite is selected from the group consisting of a glycerol and a methanol.

157. The method of claim 114, wherein the measured metabolic parameter comprises an increase or a decrease in an organic acid.

158. The method of claim 157, wherein the organic acid is selected from the group consisting of an acetate, a butyrate, a succinate and an oxaloacetate.

159. The method of claim 114, wherein the measured metabolic parameter comprises an increase or a decrease in intracellular pH.

160. The method of claim 159, wherein the increase or a decrease in intracellular pH is measured by intracellular application of a dye, and the change in fluorescence of the dye is measured over time.

161. The method of claim 114, wherein the measured metabolic parameter comprises an increase or a decrease in synthesis of DNA over time.

162. The method of claim 161, wherein the increase or a decrease in synthesis of DNA over time is measured by intracellular application of a dye, and the change in fluorescence of the dye is measured over time.

163. The method of claim 114, wherein the measured metabolic parameter comprises an increase or a decrease in uptake of a composition.

164. The method of claim 163, wherein the composition is a metabolite.

165. The method of claim 164, wherein the metabolite is selected from the group consisting of a monosaccharide, a disaccharide, a polysaccharide, a lipid, a nucleic acid, an amino acid and a polypeptide.

166. The method of claim 165, wherein the saccharide, disaccharide or polysaccharide comprises a glucose or a sucrose.

167. The method of claim 163, wherein the composition is selected from the group consisting of an antibiotic, a metal, a steroid and an antibody.

168. The method of claim 114, wherein the measured metabolic parameter comprises an increase or a decrease in the secretion of a byproduct or a secreted composition of a cell.

169. The method of claim 168, wherein the byproduct or secreted composition is selected from the group consisting of a toxin, a lymphokine, a polysaccharide, a lipid, a nucleic acid, an amino acid, a polypeptide and an antibody.

170. The method of claim 114, wherein the real time monitoring simultaneously measures a plurality of metabolic parameters.

171. The method of claim 170, wherein real time monitoring of a plurality of metabolic parameters comprises use of a Cell Growth Monitor device.

a Wedgewood Technology, Inc., Cell Growth Monitor model 652.

173. The method of claim 171, wherein the real time simultaneous monitoring measures uptake of substrates, levels of intracellular organic acids and levels of intracellular amino acids.

174. The method of claim 171, wherein the real time simultaneous monitoring measures: uptake of glucose; levels of acetate, butyrate, succinate or oxaloacetate; and, levels of intracellular natural amino acids.

175. The method of claim 171, further comprising use of a computer-implemented program to real time monitor the change in measured metabolic parameters over time.

176. The method of claim 175, wherein the computer-implemented program comprises a computer-implemented method as set forth in FIG. 28.

177. The method of claim 176, wherein the computer-implemented method comprises metabolic network equations.

178. The method of claim 176, wherein the computer-implemented method comprises a pathway analysis.

179. The method of claim 176, wherein the computer-implemented program comprises a preprocessing unit to filter out the errors for the measurement before the metabolic flux analysis.

L5 ANSWER 5 OF 112 USPATFULL on STN

2005:117615 CAPPING ENZYME OF FLAVIVIRUS AND UTILIZATION OF THIS PROTEIN IN A PROCESS TO TEST DRUGS WITH ANTIVIRAL PROPERTIES.

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US 2005100882 A1 20050512

APPLICATION: US 2002-160821 A1 20020531 (10)

PRIORITY: US 2001-294804P 20010531 (60)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention concerns an isolated and purified polypeptide capable of acting as a guanylyltransferase and methyltransferase comprising capping enzyme of flavivirus (CEF). The invention also concerns the use of such polypeptide to identify biologically active molecule which can be used in the treatment or the prevention of diseases resulting from Flavivirus infection.

CLM What is claimed is:

1. An isolated and purified capping enzyme of flavivirus (CEF) capable of acting as a guanylyltransferase and methyltransferase consisting essentially of a polypeptide from the N-terminus of flavivirus genus RNA-dependent RNA polymerase.

2. The capping enzyme of flavivirus (CEF) according to claim 1, wherein said polypeptide has a three-dimensional structure and comprises a N-terminal module (subdomain 1), a SAM-binding core (subdomain 2), and a C-terminal sequence (subdomain 3) located between subdomains 1 and 2 in the three-dimensional structure of the polypeptide, and forming a bottom portion of a narrow cleft.

3. The capping enzyme of flavivirus (CEF) according to claim 2, wherein said subdomain 1 starts with a helix A1-turn-helix A2 motif.

4. The capping enzyme of flavivirus (CEF) according to claim 2, wherein said subdomain 2 comprises a twisted mixed β -sheet comprising 7 β -strands and 5 helices.

5. The capping enzyme of flavivirus (CEF) according to claim 2, wherein said subdomain 3 is positively charged.

6. The capping enzyme of flavivirus (CEF) according to claim 1, wherein said CEF is a polypeptide comprising SEQ ID No. 1.

7. The capping enzyme of flavivirus (CEF) according to claim 1, wherein said CEF is a polypeptide comprising SEQ ID No. 2.

8. The capping enzyme of flavivirus (CEF) according to claim 1, wherein said CEF is a polypeptide comprising SEQ ID No. 3.

9. A nucleic acid molecule comprising an encoding nucleic sequence for a polypeptide capable of acting as a guanylyltransferase and

according to claim 1.

10. A polyclonal or monoclonal antibody directed against a capping enzyme of flavivirus (CEF) according to claim 1, or a derivative or a fragment of said antibody.

11. A vector comprising at least one molecule of nucleic acid according to claim 9, and control sequences.

12. A cellular host transformed by one molecule of nucleic acid according to claim 9.

13. A cellular host transformed by a vector according to claim 11.

14. A nucleic or **oligonucleotide** probe prepared from one molecule of nucleic acid according to claim 9.

15. A method for determining inhibitory power of a biologically active compound acting as a competitive inhibitor of GTP comprising: a) incubating CEF according to claim 1 with radiolabeled GTP; b) adding selected different concentrations of said biologically active compound; c) assaying resulting radiolabeled CEF-GTP complex produced from step a); d) quantifying an amount of radiolabeled CEF-GTP complex produced from step c) e) comparing said amount to a binding affinity constant of GTP to CEF; and f) determining said inhibitory power of said biologically active compound.

16. The method according to claim 15, wherein the concentration at which said incubating is conducted at about 52 μ M of GTP.

17. The method according to claim 16, wherein said selected different concentrations of GTP are selected from the group consisting of about 0 μ M, about 10 μ M, about 20 μ M, about 50 μ M, about 100 μ M, about 200 μ M, about 300 μ M, about 500 μ M, and about 800 μ M.

18. The method according to claim 15, wherein said assaying comprises UV-crosslinking α -³²P-GTP to CEF.

19. A method for selecting an inhibitory biologically active compound capable of reducing CEF binding to GTP as an antiviral pharmaceutical agent according to claim 15, further comprising: selecting biologically active compounds with a binding affinity higher than the binding affinity constant of GTP to CEF.

20. A method according to claim 15, wherein the biologically active compound is a nucleoside or nucleoside analogue.

21. A method of treating diseases resulting from Flavivirus infection comprising administering a therapeutically effective amount of a composition formed from acyclovir 5'-triphosphate or a vectorized form of acyclovir 5'-monophosphate to a patient.

22. A method preventing diseases resulting from Flavivirus infection comprising administering a therapeutically effective amount of a composition formed from acyclovir 5'-triphosphate or a vectorized form of acyclovir 5'-monophosphate to a patient.

23. Use of acyclovir 5'-triphosphate or a vectorized form of acyclovir 5'-monophosphate for the preparation of a medicine useful in the treatment or the prevention of diseases resulting from Flavivirus infection.

24. The isolated and purified capping enzyme of flavivirus (CEF) of claim 1, wherein the polypeptide is about 33 kDa.

25. The isolated and purified capping enzyme of flavivirus (CEF) of claim 1, wherein the RNA-dependent RNA polymerase is a Dengue, Yellow Fever or West Nile virus RNA-dependent RNA polymerase.

L5 ANSWER 6 OF 112 USPTAFULL on STN

2005:44506 Novel compositions.

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US 2005038239 A1 20050217

APPLICATION: US 2004-480424 A1 20040614 (10)

WO 2002-GB2728 20020614

PRIORITY: GB 2001-14719 20010615

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to compositions comprising DNA attached to one or more functional moieties via a locked nucleic acid

compositions comprising a plasmid containing a gene encoding a protein of interest, wherein said plasmid may be introduced to a tissue or cell and the gene expressed, complexed to the locked nucleic acid functional moiety

CLM What is claimed is:

1. A locked nucleic acid conjugate comprising an **oligonucleotide** comprising at least one locked nucleic acid base and a functional moiety.
2. A locked nucleic acid conjugate as claimed in claim 1, wherein the functional moiety is a member selected from the group consisting of: Fluorescent labels; nuclear localisation peptides; peptides that have the ability to cross the plasma membrane of eukaryotic cells ("cell penetrating peptides"); endosomal escape peptides; cell targeting and binding peptides or protein; peptides or proteins with transcription activation domains; and molecules having adjuvant or immunostimulatory activity.
3. A locked nucleic acid conjugate as claimed in claim 1 wherein the **oligonucleotide** is between 7 to 25 bases in length.
4. A locked nucleic acid conjugate as claimed in claim 1 wherein at least 50% of the bases are locked nucleic acid bases.
5. A locked nucleic acid conjugate as claimed in claim 1 wherein the at least one locked nucleic acid base is a member selected from the group consisting of O--, (oxy) S--, (thio) ad NH--2 (amino) bridged locked nucleic acid base.
6. A locked nucleic acid conjugate as claimed in claim 1 wherein the **oligonucleotide** is free from self-complementary base pairings.
7. A locked nucleic acid conjugate as claimed in claim 1 comprising a cleavable linkage between the functional moiety and the **oligonucleotide** which is selectively cleavable after administration to a patient.
8. A locked nucleic acid conjugate as claimed in claim 1 wherein the functional moiety is an immunostimulatory **oligonucleotide** containing at least one unmethylated CG di-nucleotide motif.
9. A complex comprising a locked nucleic acid conjugate as claimed in claim 1, and a DNA sequence having a complementary sequence to the **oligonucleotide**, and encoding a gene under the control of a promoter.
10. A complex as claimed in claim 9 wherein at least one further locked nucleic acid conjugate is present which is bound to a complementary sequence within the locked nucleic acid complex which is itself bound to the DNA sequence.
11. A complex as claimed in claim 10 comprising an array of locked nucleic acid conjugates bound to a single locked nucleic acid complementary sequence within the DNA, formed by locked nucleic acid: locked nucleic acid hybridisation between locked nucleic acid **oligonucleotide**.
12. A complex as claimed in claim 9 wherein the gene encodes for a therapeutic protein or an antigen.
13. A complex as claimed in claim 10 wherein a plurality of locked nucleic acid complexes are bound to a plurality of complementary sequences within said DNA sequence.
14. A complex of claim 12 wherein the antigen is capable of raising an immune response against a pathogen or a tumour.
15. A complex as claimed in claim 9 wherein the DNA sequence is in the form of an open circular or supercoiled plasmid.
16. A pharmaceutical composition comprising a complex as claimed in claim 9 and a pharmaceutically acceptable carrier or diluent.
17. A pharmaceutical composition as claimed in claim 16 wherein the complex is coated on to a microprojectile.
18. A pharmaceutical composition as claimed in claim 17 wherein the microprojectiles are gold beads.
19. A device loaded with the pharmaceutical composition of claim 16.
- 20-23. cancelled.

comprising the step of hybridising the locked nucleic acid conjugate of claim 1 with a plasmid capable of expressing a gene encoding an antigen or therapeutic protein, and formulating the resulting complex with a pharmaceutical acceptable carrier.

25. An **oligonucleotide** comprising a first region comprising an **oligonucleotide** sequence having at least one locked nucleic acid, and a second region comprising an immunostimulatory **oligonucleotide** region containing at least one unmethylated CG di-nucleotide motif.

26. An **oligonucleotide** as claimed in claim 25 wherein the first locked nucleic acid containing region and the second immunostimulatory **oligonucleotide** region are separated by a phosphoramidate region, and wherein the second immunostimulatory **oligonucleotide** region comprises a phosphorothioate backbone.

L5 ANSWER 7 OF 112 USPATFULL on STN

2004:235351 Flavivirus detection and quantification assay.

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US 6793488 B1 20040921

APPLICATION: US 2000-551161 20000414 (9)

PRIORITY: US 1999-153685P 19990914 (60)

US 1999-129713P 19990416 (60)

DOCUMENT TYPE: Utility; GRANTED.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A fluorescent DNA probes specific to the conserved terminal 3'-noncoding region (nucleotides 10653-10678) of **dengue virus** and a pair of flanking primers are designed to formulate a dengue specific fluorogenic **polymerase chain reaction (PCR)**. Optimal assay conditions with zero background are disclosed which permit the detection of low levels of **dengue virus** from clinical specimens. **Dengue virus** isolates from different geographic regions can be universally detected and identified by the fluorogenic RT-**PCR** assay. Moreover, the assay is specific for dengue 2 virus and does not recognize other related flaviviruses, including dengue serotypes Louis encephalitis, yellow fever, and Kunjin viruses. The) 3, and 4, Japanese encephalitis, St. assay also efficiently detected immunocomplexed dengue viruses. The fluorogenic RT-**PCR** assay readily detected viremia in sera collected from individuals ill with dengue fever.

CLM What is claimed is:

1. A composition suitable for use in a process involving DNA amplification comprising DNA segments of 19-36 nucleotides in length which have sequences selected from: Serotype-specific Upstream **Primers** (5) DV1-1U 5'-GAT-CAA-GCT-TACA-CCA-GGG-GAA-GCT-GTA-TCC-TGG-3' (SEQ ID NO 4), DV2-2U 5'-GAT-CAA-GCT-TAAG-GTC-AGA-TGA-AGC-TGT-AGT-CTC-3' (SEQ ID NO 5), DV3-1U 5'-GAT-CAA-GCT-TAGC-ACT-GAG-GGA-AGC-TGT-ACC-TCC-3' (SEQ ID NO 6), DV4-1U 5'-GAT-CAA-GCT-TAAG-CCA-GGA-GGA-AGC-TGT-ACT-CCT-3' (SEQ ID NO 7), and JE.F2-5'-CAAGCCCCCTCGAAGCTGT-3' (SEQ ID NO 13); Serotype-specific Fluorescent probes (4) DV1-P1 5'-CTG-TCT-DTA-CAG-CAT-CAT-TCC-AGG-CA-3' (SEQ ID NO 8), DV4-P1 5'-CTG-TCT-CTG-CAA-CAT-CAA-TCC-AGG-CA-3' (SEQ ID NO 9), DV2-P1 5'-CTG-TCT-CCT-CAG-CAT-CAT-TCC-AGG-CA-3' (SEQ ID NO 1), and JE.P1 5'-TCTGCTCTATCTCAACATCAGCTACTAGGCACAGA-3' (SEQ ID NO 12); and Serotype-specific Down-stream **Primer** (3) DV4-1L 5'-CAA-TCC-ATC-TTG-CGG-CGC-TCT-3' (SEQ ID NO 10), DV2-1L 5'-GAT-CGA-ATT-CCAT-TCC-ATT-TTC-TGG-CGT-TCT-3' (SEQ ID NO 11), and JE.R382 5'-CACCAGTACATACTTCGGCG-3' (SEQ ID NO 14); or the complement thereof, wherein the composition contains a) at least one of said serotype-specific upstream **primers** and one of said serotype-specific down stream **primers** or b) one of said serotype-specific fluorescent probes.

2. The isolated DNA segments of claim 1 wherein said segments are probes and are labeled.

3. The probe of claim 2 wherein the label is fluorescent.

4. The probe of claim 2 wherein the label is a quencher.

5. The probe of claim 2 wherein the segment is labeled at both the 3' and 5' end, respectively, where one label is a quencher and the other is a fluorescent.

6. A **PCR**-based diagnostic kit for detecting or quantitating a flavivirus serotype comprising: isolated DNA segments of 19-36 nucleotides which have sequences selected from: Serotype-specific Upstream **Primers** (5) DV1-1U 5'-GAT-CAA-CT-TACA-CCA-GGG-GAA-GCT-GTA-TCC-TGG-3' (SEQ ID NO 4), DV2-2U 5'-GAT-CAA-GCT-TAAG-GTC-AGA-TGA-AGC-TGT-AGT-CTC-3' (SEQ ID NO 5), DV3-1U 5'-GAT-CAA-GCT-TAGC-ACT-GAG-GGA-AGC-TGT-ACC-

CCT-3' (SEQ ID NO 7), and JE.F214 5'-CAAGCCCCCTCGAAGCTGT-3' (SEQ ID NO 13); Serotype-specific Fluorescent probes (4) DV1-P1 5'-CTG-TCT-DTA-CAG-CAT-CAT-TCC-AGG-CA-3' (SEQ ID NO 8), DV4-P1 5'-CTG-TCT-CTG-CAA-CAT-CAA-TCC-AGG-CA-3' (SEQ ID NO 9), DV2-P1 5'-CTG-TCT-CCT-CAG-CAT-CAT-TCC-AGG-CA-3' (SEQ ID NO 1), and JE.P1 5'-TCTGCTCTATCTCAACATCAGCTACTAGGCACAGA-3' (SEQ ID NO 12); and Serotype-specific Down-stream **Primer** (3) DV4-1L 5'-CAA-TCC-ATC-TTG-CGG-CGC-TCT-3' (SEQ ID NO 10), DV2-1L 5'-GAT-CGA-ATT-CCAT-TCC-ATT-TTC-TGG-CGT-TCT-3' (SEQ ID NO 11), and JE.R382 5'-CACCAGCTACATACTTCGGCG-3' (SEQ ID NO 14); or the complement thereof, wherein the kit contains a) at least one of said serotype-specific upstream **primers** and one of said serotype-specific down stream **primers** or b) one of said serotype-specific fluorescent probes.

7. The kit of claim 6 wherein said segments are labeled probes.

8. The kit of claim 7 wherein the label is fluorescent.

9. The kit of claim 7 wherein the label is a quencher.

10. The kit of claim 6 wherein the segment is labeled at both the 3' and 5' end, respectively, where one label is a quencher and the other is a fluorescent.

11. A method for detecting or quantifying one or more species of flavivirus contained in sample comprising the steps of: i) collecting a sample suspected of containing a flavivirus; ii) preparing said sample for **PCR** amplification; iii) adding to said prepared sample, **PCR** reagents including both probes and **primer** pairs wherein the probes and **primers** in the **primer** pair consist of 19-36 nucleotides in length and contain any one of the following, Serotype-specific Upstream **Primers** (5) DV1-1U 5'-GAT-CAA-GCT-TACA-CCA-GGG-GAA-GCT-GTA-TCC-TGG-3' (SEQ ID NO 4), DV2-2U 5'-GAT-CAA-GCT-TAAG-GTC-AGA-TGA-AGC-TGT-AGT-CTC-3' (SEQ ID NO 5), DV3-1U 5'-GAT-CAA-GCT-TAGC-ACT-GAG-GGA-AGC-TGT-ACC-TCC-3' (SEQ ID NO 6), DV4-1U 5'-GAT-CAA-GCT-TAAG-CCA-GGA-GGA-AGC-TGT-ACT-CCT-3' (SEQ ID NO 7), and JE.F214 5'-CAAGCCCCCTCGAAGCTGT-3' (SEQ ID NO 13); Serotype-specific Fluorescent probes (4) DV1-P1 5'-CTG-TCT-DTA-CAG-CAT-CAT-TCC-AGG-CA-3' (SEQ ID NO 8), DV4-P1 5'-CTG-TCT-CTG-CAA-CAT-CAA-TCC-AGG-CA-3' (SEQ ID NO 9), DV2-P1 5'-CTG-TCT-CCT-CAG-CAT-CAT-TCC-AGG-CA-3' (SEQ ID NO 1), and JE.P1 5'-TCTGCTCTATCTCAACATCAGCTACTAGGCACAGA-3' (SEQ ID NO 12); and Serotype-specific Down-stream **Primer** (3) DV4-1L 5'-CAA-TCC-ATC-TTG-CGG-CGC-TCT-3' (SEQ ID NO 10), DV2-1L 5'-GAT-CGA-ATT-CCAT-TCC-ATT-TTC-TGG-CGT-TCT-3' (SEQ ID NO 11), and JE.R382 5'-CACCAGCTACATACTTCGGCG-3' (SEQ ID NO 14); or the complement thereof, wherein a) the **primer** pairs comprise at least one of said serotype-specific upstream **primers** and one of said serotype-specific down stream **primers** or b) at least one probe is one of said serotype-specific fluorescent probes; iv) maintaining the sample under conditions suitable for amplification; v) detecting or quantifying one or more of the flavivirus species.

12. The method of claim 11 wherein said serotype-specific fluorescent probes of step (iii) are labeled with a fluorescent label.

13. The method of claim 11 wherein said serotype-specific fluorescent probes of step (iii) are labeled with a quencher.

14. The method of claim 11 wherein the segment is labeled at both the 3' and 5' end, respectively, where one label is a quencher and the other is a fluorescent.

15. The method of claim 11 wherein said flavivirus is Dengue.

16. The method of claim 15 wherein said **Dengue virus** is Dengue 1, 2, 3, or 4.

17. A method for detecting or quantifying **dengue virus** Serotype(s) comprising i) contacting a sample suspected of containing a flavivirus with **PCR** reagents, including at least two **PCR primers** selected from the following groups: Serotype-specific Upstream **Primers** (5) DV1-1U 5'-GAT-CAA-GCT-TACA-CCA-GGG-GAA-GCT-GTA-TCC-TGG-3' (SEQ ID NO 4), DV2-2U 5'-GAT-CAA-GCT-TAAG-GTC-AGA-TGA-AGC-TGT-AGT-CTC-3' (SEQ ID NO 5), DV3-1U 5'-GAT-CAA-GCT-TAGC-ACT-GAG-GGA-AGC-TGT-ACC-TCC-3' (SEQ ID NO 6), DV4-1U 5'-GAT-CAA-GCT-TAAG-CCA-GGA-GGA-AGC-TGT-ACT-CCT-3' (SEQ ID NO 7), and JE.F214 5'-CAAGCCCCCTCGAAGCTGT-3' (SEQ ID NO 13); Serotype-specific Down-stream **Primer** (3) DV4-1L 5'-CAA-TCC-ATC-TTG-CGG-CGC-TCT-3' (SEQ ID NO 10), DV2-1L 5'-GAT-CGA-ATT-CCAT-TCC-ATT-TTC-TGG-CGT-TCT-3' (SEQ ID NO 11), and JE.R382 5'-CACCAGCTACATACTTCGGCG-3' (SEQ ID NO 14), or the complement thereof, wherein the **primer** pairs comprise at least one of said serotype specific upstream **primers** and one of said serotype specific down stream **primers**, and a polymerase enzyme, and an **oligonucleotide** probe selected from the following

5'-CTG-TCT-DTA-CAG-CAT-CAT-TCC-AGG-CA-3' (SEQ ID NO 8), DV4-P1
 5'-CTG-TCT-CTG-CAA-CAT-CAA-TCC-AGG-CA-3' (SEQ ID NO 9), DV2-P1
 5'-CTG-TCT-CCT-CAG-CAT-CAT-TCC-AGG-CA-3' (SEQ ID NO 1), and JE.P1
 5'-TCTGCTCTATCTCAACATCAGCTACTAGGCACAGA-3' (SEQ ID NO 12), or the complement thereof, wherein at least one of said **oligonucleotide** probes is a serotype specific fluorescent probe, and wherein a fluorescer molecule attached to a first end of the **oligonucleotide** probe and a quencher molecule attached to a second end of the **oligonucleotide** probe such that the quencher molecule substantially quenches the fluorescer molecule whenever the **oligonucleotide** probe is in the free stranded state and such that the fluorescer is substantially unquenched whenever the **oligonucleotide** probe is hybridized to the target nucleic acid; a 5' end which is rendered impervious to digestion by the 5'→3' exonuclease activity of a polymerase; and a 3' end which is rendered impervious to the 5'→3' extension activity of the polymerase; and ii) subjecting the sample, **oligonucleotide** probe, and the **PCR** reagents to thermal cycling, including a polymerization step, the thermal cycling being sufficient to amplify the target nucleic acid specified by the **PCR** reagents.

18. The method of claim 17 further comprising the step of measuring the extent of fluorescence quenching of the **oligonucleotide** probe, such measurement being performed subsequent to thermocycling and at a probe hybridization temperature.

19. The method of claim 17 further comprising the step of measuring the extent of fluorescence quenching of the **oligonucleotide** probe at a probe hybridization temperature in a manner which locates the probe within the individual cells originally containing the target nucleic acid sequence.

20. The method of claim 17 wherein the sample, the **PCR** reagents, and the **oligonucleotide** probe are located in a containment assembly.

21. The method of claim 17 wherein the probe hybridization temperature is less than or equal to the temperature of the polymerization step of the thermocycling.

L5 ANSWER 8 OF 112 USPTAFULL on STN

2004:190965 Novel anti-infectives.

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 US 2004147739 A1 20040729

APPLICATION: US 2003-479358 A1 20031202 (10)

WO 2002-US18491 20020607

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Compounds useful as HCV anti-infectives having the formula: wherein the formula variables are as defined herein, are disclosed. Also disclosed are methods of making and using the same.

CLM What is claimed is:

1. A compound according to Formula I: ##STR12## wherein: R¹ is hydrogen, halogen, C₁₋₄ alkyl, --OR¹¹, --SR¹¹, --NR^{10R11}, aryl, --C(O)OH, --C(O)NHR¹¹, cyano or nitro; R² is hydrogen, C₁₋₈ alkyl, C₂₋₈ alkenyl, C₂₋₈ alkynyl, C₃₋₆ cycloalkyl, heterocycloalkyl, aryl, heteroaryl, nitro, cyano, halogen, --C(O)OR⁹, --C(O)R⁹, --C(O)NR^{9R10}, --OR⁹, --SR⁹, --S(O)R¹², --S(O)₂R¹², --NR^{9R10}, protected --OH, --N(R^{10C}(O)R⁹, --OC(O)NR^{9R10}, --N(R¹⁰)C(O)NR^{9R10}, --P(O)(OR⁹)₂, --SO₂NR^{9R10}, --SO₃H, or --N(R¹⁰)SO₂R¹², where said C₁₋₈ alkyl, C₂₋₈ alkenyl or C₂₋₈ alkynyl is unsubstituted or substituted with one or more substituents independently selected from halogen, --OH, --SH, --OC₁₋₄ alkyl, --SC₁₋₄ alkyl, --NR^{10R11}, cyano, nitro, --CO₂R¹⁰, --C(O)OC₁₋₄ alkyl, --CONR^{10R11}, --CONH₂, aryl,

heteroaryl is unsubstituted or substituted with one or more substituents independently selected from C₁-C₆ alkyl, C₁-C₆ haloalkyl, halogen, --OH, --SH, --NH₂, --OC₁-C₄ alkyl, --SC₁-C₄ alkyl, --N(C₁-C₄ alkyl)(C₁-C₄ alkyl), --NH(C₁-C₄ alkyl), cyano, nitro, --CO₂H, --C(O)OC₁-C₄ alkyl, --CON(C₁-C₄ alkyl)(C₁-C₄ alkyl), --CONH(C₁-C₄ alkyl) and --CONH₂; R³ is hydrogen, halogen, cyano, C₁-C₆ alkyl, --OH, or --CO₂H; R⁴, R⁵ and R⁶ are each independently selected from the group consisting of hydrogen, halogen, cyano, C₁-C₆ alkyl, --OH, and --OC₁-C₄ alkyl; R⁷ is hydrogen, C₁-C₈ alkyl, C₂-C₈ alkenyl, C₂-C₈ alkynyl, C₃-C₆ cycloalkyl, heterocycloalkyl, aryl, heteroaryl, nitro, cyano, halogen, --C(O)OR⁹, --C(O)R⁹, --C(O)NR⁹R¹⁰, --OR⁹, --SR⁹, (O)R¹², --S(O)₂R¹², NR⁹R¹⁰, protected --OH, --N(R¹⁰)C(O)R⁹, --OC(O)NR⁹R¹⁰, --N(R¹⁰)C(O)NR⁹R¹⁰, --P(O)(OR⁹)₂, --SO₂NR⁹R¹⁰, --SO₃H, or --N(R¹⁰)SO₂R¹², where said C₁-C₈ alkyl, C₂-C₈ alkenyl or C₂-C₈ alkynyl is unsubstituted or substituted with one or more substituents independently selected from halogen, --OH, --SH, --OC₁-C₄ alkyl, --SC₁-C₄ alkyl, --NR¹⁰R¹¹, cyano, nitro, --CO₂H, --C(O)OC₁-C₄ alkyl, --CONR¹⁰R¹¹, --CONH₂, aryl, heteroaryl, heterocycloalkyl, --C(O)aryl, --C(O)heterocycloalkyl, and --C(O)heteroaryl, where said aryl, heteroaryl, heterocycloalkyl, aryl, --C(O)aryl, --C(O)heterocycloalkyl, or --C(O)heteroaryl is unsubstituted or substituted with one or more substituents independently selected from C₁-C₄ alkyl, C₁-C₄ haloalkyl, halogen, --OH, --SH, --NH₂, --OC₁-C₄ alkyl, --SC₁-C₄ alkyl, --N(C₁-C₄ alkyl)(C₁-C₄ alkyl), --NH(C₁-C₄ alkyl), cyano and nitro, and where said cycloalkyl, heterocycloalkyl, aryl or heteroaryl is unsubstituted or substituted with one or more substituents independently selected from C₁-C₆ alkyl, C₁-C₆ haloalkyl, halogen, --OH, --SH, --NH₂, --OC₁-C₄ alkyl, --SC₁-C₄ alkyl, --N(C₁-C₄ alkyl)(C₁-C₄ alkyl), --NH(C₁-C₄ alkyl), cyano, nitro, --CO₂H, --C(O)OC₁-C₄ alkyl, --CON(C₁-C₄ alkyl)(C₁-C₄ alkyl), --CONH(C₁-C₄ alkyl) and --CONH₂; R⁸ is hydrogen, halogen, hydroxyl or C₁-C₄ alkyl; or R¹ and R² or R⁵ and R⁶ or R⁶ and R⁷ or R⁷ and R⁸ taken together are alkylenedioxy; W is hydrogen, --C(O)OR¹¹, C₁-C₁₀ alkyl, C₂-C₁₀ alkenyl, C₂-C₁₀ alkynyl, C₃-C₆ cycloalkyl, --(C₁-C₆ alkyl)-(C₃-C₆ cycloalkyl), --(C₂-C₆ alkenyl)-(C₃-C₆ cycloalkyl), --(C₂-C₆ alkynyl)-(C₃-C₆ cycloalkyl), --(C₁-C₆ alkyl)-heterocycloalkyl, --(C₂-C₆ alkenyl)-heterocycloalkyl, --(C₂-C₆ alkynyl)-heterocycloalkyl, --(C₁-C₆ alkyl)-aryl, (C₂-C₆ alkenyl)-aryl, --(C₂-C₆ alkynyl)-aryl, --(C₁-C₆ alkyl)-heteroaryl, --(C₂-C₆ alkenyl)-heteroaryl, or --(C₂-C₆ alkynyl)-heteroaryl, where said C₁-C₁₀ alkyl, C₂-C₁₀ alkenyl, C₂-C₁₀ alkynyl is unsubstituted or substituted with one or more substituents independently selected from halogen, cyano, --OH, --OC₁-C₄ alkyl, --SH, --SC₁-C₄ alkyl, --S(O)(C₁-C₄ alkyl), --SO₃H, and --S(O)₂(C₁-C₄ alkyl), said C₃-C₆ cycloalkyl is unsubstituted or substituted with one or more substituents independently selected from halogen, cyano, C₁-C₄ alkyl, --OH, --OC₁-C₄ alkyl, --SH, --SC₁-C₄ alkyl, --S(O)(C₁-C₄ alkyl), --SO₃H, and --S(O)₂(C₁-C₄ alkyl), and where the cycloalkyl, heterocycloalkyl, aryl or heteroaryl moiety of said --(C₁-C₆ alkyl)-(C₃-C₆ cycloalkyl), --(C₂-C₆ alkenyl)-(C₃-C₆ cycloalkyl), --(C₂-C₆ alkynyl)-(C₃-C₆ cycloalkyl), --(C₁-C₆ alkyl)-heterocycloalkyl, --(C₂-C₆ alkenyl)-heterocycloalkyl, --(C₂-C₆ alkynyl)-heterocycloalkyl, --(C₁-C₆ alkyl)-aryl, (C₂-C₆ alkenyl)-aryl, --(C₂-C₆ alkynyl)-aryl, --(C₁-C₆ alkyl)-heteroaryl, --(C₂-C₆ alkenyl)-heteroaryl, or --(C₂-C₆ alkynyl)-heteroaryl is unsubstituted or substituted with one or more substituents independently selected from C₁-C₆ alkyl, C₁-C₆ haloalkyl, halogen, cyano, nitro, --OH, --NH₂, --OC₁-C₄ alkyl, --N(C₁-C₄ alkyl)(C₁-C₄ alkyl), and --NH(C₁-C₄ alkyl); X is O or S; Y is --OH or --SH; Z is hydrogen or C₁-C₄ alkyl; wherein each R⁹ is

C₁-c₈ alkyl, C₂-c₈ alkenyl, C₂-c₈ alkynyl,
C₃-c₈ cycloalkyl, heterocycloalkyl, aryl, heteroaryl,
--C₁-c₆ alkyl-C₃-c₈ cycloalkyl, --C₁-c₆
alkyl-heterocycloalkyl, --C₁-c₆ alkyl-aryl, and
--C₁-c₆ alkyl-heteroaryl, --C₂-c₆
alkenyl-C₃-c₈ cycloalkyl, --C₂-c₆
alkenyl-heterocycloalkyl, --C₂-c₆ alkenyl-aryl,
--C₂-c₆ alkenyl-heteroaryl, --C₂-c₆
alkynyl-C₃-c₈ cycloalkyl, --C₂-c₆
alkynyl-heterocycloalkyl, --C₂-c₆ alkynyl-aryl, and
--C₂-c₆ alkynyl-heteroaryl, where said C₁-c₈ alkyl,
C₂-c₈ alkenyl, or C₂-c₈ alkynyl is unsubstituted or
substituted with one or more substituents independently selected from
halogen, --OR¹¹, --NR^{10R11}, cyano, nitro,
--CO_{2R}¹¹, --CONR^{10R11}, --
NR^{10CONR10R11}, --OCONR^{10R11},
--SO_{2NR}^{10R11}, and --COR¹¹, and where any of said
cycloalkyl, heterocycloalkyl, aryl or heteroaryl (including the
cycloalkyl, heterocycloalkyl, aryl or heteroaryl moieties of said
--C₁-c₆ alkyl-C₃-c₈ cycloalkyl, --C₁-c₆
alkyl-heterocycloalkyl, --C₁-c₆ alkyl-aryl, or
--C₁-c₆ alkyl-heteroaryl) is unsubstituted or substituted with
one or more substituents independently selected from C₁-c₄
alkyl, C₁-c₄ haloalkyl, halogen, --OR¹¹,
--NR^{10R11}, cyano, nitro, --CO_{2R}¹¹,
--CONR^{10R11}, --NR^{10CONR10R11},
--OCONR^{10R11}, --SO_{2NR}^{10R11}, and --COR¹¹;
each R¹⁰ is independently selected from hydrogen and
C₁-c₆ alkyl; each R¹¹ is independently selected from the
group consisting of hydrogen, C₁-c₆ alkyl, C₃-c₆
cycloalkyl, heterocycloalkyl, aryl, heteroaryl, --C₁-c₄
alkyl-C₃-c₈ cycloalkyl, --C₁-c₄ alkyl-
heterocycloalkyl, --C₁-c₄ alkyl-aryl, or --C₁-c₄
alkyl-heteroaryl where said cycloalkyl, heterocycloalkyl, aryl,
heteroaryl, -alkylcycloalkyl, -alkylheterocycloalkyl, -alkylaryl or
-alkylheteroaryl is unsubstituted or substituted with one or more
substituents independently selected from C₁-c₆ alkyl,
C₁-c₆ haloalkyl, halogen --OC₁-c₆ alkyl,
--OC₁-c₆ haloalkyl, cyano, --N(C₁-c₆
allyl)(C₁-c₆ alkyl), --NH(C₁-c₆ alkyl), --NH₂,
--CO_{2C1}-c₆ alkyl, --CO_{2H}, --CON(C₁-c₆
alkyl)(C₁-c₆ alkyl), --CONH(C₁-c₆ allyl), and
--CONH₂; or, when present in any NR^{9R10} or
NR^{10R11}, each R⁹ and R¹⁰ or each R¹⁰ and
R¹¹, independently, taken together with the nitrogen to which they
are attached represent a 3-6-membered saturated ring optionally
containing one other heteroatom selected from oxygen and nitrogen, where
said 3-6-membered ring is unsubstituted or substituted with one or more
substituents independently selected from hydrogen, C₁-c₆
alkyl, halogen, cyano, --OC₁-c₆ alkyl, --OH,
--N(C₁-c₆ alkyl)(C₁-c₆ alkyl), --NH(C₁-c₆
alkyl), --NH₂, --CO_{2H}, --C(O)OC₁-c₆ alkyl,
--C(O)C₁-c₆ alkyl, --CON(C₁-c₆ alkyl)(C₁-
c₆ alkyl), --CONH(C₁-c₆ alkyl), --CONH₂,
C₃-c₆ cycloalkyl, heterocycloalkyl, aryl, heteroaryl,
C₃-c₆ cycloalkyl-C₁-c₆ alkyl-, heterocycloalkyl-
C₁-c₆ alkyl-, aryl-C₁-c₆ alkyl- and
heteroaryl-C₁-c₆ alkyl-, and where said cycloalkyl,
heterocycloalkyl, aryl, heteroaryl, cycloalkylalkyl-,
heterocycloalkylalkyl-, arylalkyl- or heteroarylalkyl- is unsubstituted
or substituted with one or more substituents independently selected from
C₁-c₆ alkyl, C₁-c₆ haloalkyl, halogen
--OC₁-c₆ alkyl, --OC₁-c₆ haloalkyl, cyano,
--N(C₁-c₆ alkyl)(C₁-c₆ alkyl), --NH(C₁-c₆
alkyl), --NH₂, --CO_{2C1}-c₆ alkyl, --CO_{2H},
--CON(C₁-c₆ alkyl)(C₁-c₆ alkyl),
--CONH(C₁-c₆ alkyl), and --CONH₂; each R¹² is
independently selected from the group consisting of C₁-c₈
alkyl, C₂-c₈ alkenyl, C₂-c₈ alkynyl, C₃-c₈
cycloalkyl, heterocycloalkyl, aryl, heteroaryl, --C₁-c₆
alkyl-C₃-c₈ cycloalkyl, --C₁-c₆ alkyl-
heterocycloalkyl, --C₁-c₆ alkyl-aryl, and --C₁-c₆
alkyl-heteroaryl, --C₂-c₆ alkenyl-C₃-c₈ cycloalkyl,
--C₂-c₆ alkenyl-heterocycloalkyl, --C₂-c₆
alkenyl-aryl, --C₂-c₆ alkenyl-heteroaryl, --C₂-c₆
alkynyl-C₃-c₈ cycloalkyl, --C₂-c₆
alkynyl-heterocycloalkyl, --C₂-c₆ alkynyl-aryl, and
--C₂-c₆ alkynyl-heteroaryl, where said C₁-c₈ alkyl,

substituted with one or more substituents independently selected from halogen, --OR¹³, --NR^{10R13}, cyano, nitro, --O_{2R}^{10R13}, --CONR^{10R13}, --NR^{10CONR10R13}, --OCONR^{10OR13}, --SO_{2NR}^{10R13}, and --COR¹³, and where any of said cycloalkyl, heterocycloalkyl, aryl or heteroaryl is unsubstituted or substituted with one or more substituents independently selected from C₁-c₄ alkyl, C₁-c₄ haloalkyl, halogen, --OR¹³, --NR^{10R13}, cyano, nitro, --CO_{2R}¹³, --CONR^{10R13}, --NR^{10CONR10R13}, --OCONR^{10R13}, --SO_{2NR}^{10R13}, and --COR¹³; each R¹³ is independently selected from the group consisting of hydrogen, C₁-c₈ alkyl, C₂-c₈ alkenyl, C₂-c₈ alkynyl, C₃-c₈ cycloalkyl, heterocycloalkyl, aryl, heteroaryl, --C₁-c₆ alkyl-C₃-c₈ cycloalkyl, --C₁-c₆ alkyl-heterocycloalkyl, --C₁-c₆ alkyl-aryl, and --C₁-c₆ alkyl-heteroaryl; provided that when X is O, Y is OH and Z, R¹, R², R³, R⁴, R⁵, R⁶, R⁷ and R⁸ are hydrogen: W is not hydrogen, --CH₃, --C₂H₅, --nC₃H₇, --nC₄H₉, --nC₅H₁₁, --nC₆H₁₃, --nC₇H₁₅, --(CH₂)CH(CH₃)₂, --(CH₂)₂CH(CH₃)₂, --CH₂CH.dbd.CH₂, --CH₂CH.dbd.CH(CH₃), --(CH₂)₃CN, --(CH₂)₄CN, --(CH₂)phenyl, --(CH₂)pyridin-2-yl, or --(CH₂)₂₀CH₃; or a tautomer thereof, or a pharmaceutically acceptable salt or solvate thereof.

2. The compound according to claim 1, wherein: R¹ is hydrogen, halogen, C₁-c₄ alkyl, aryl, --OR^a, --C(O)OR⁶, --C(O)NR^{aRa} or cyano; R² is hydrogen, C₁-c₆ alkyl, C₁-c₆ haloalkyl, aryl, heteroaryl, nitro, cyano, halogen, --C(O)OR^a, --C(O)C₁-c₆ alkyl, --C(O)NR^{aRa}, --OR^b, protected --OH, --SR^b, --S(OR)^c, --S(O)_{2R}^b, --NR^{aRc}, --NR^{aC}(O)C₁-c₆ alkyl, --NR^{aC}Oaryl, --NR^{aC}(C₁-c₄ alkyl)aryl, --NR^{aC}(O)heteroaryl, --NR^{aC}(O)(C₁-c₄ alkyl)heteroaryl, --NR^{aC}(O)cycloalkyl, --NR^{aC}(O)(C₁-c₄ alkyl)cycloalkyl, --NR^{aC}(O)heterocycloalkyl, --NR^{aC}(O)(C₁-c₄ alkyl)heterocycloalkyl, where each of said C₁-c₆ alkyl is optionally unsubstituted or substituted by one or more substituents independently selected from the group consisting of cyano, --OC₁-c₄ alkyl, --OH, --N(C₁-c₄ alkyl)(C₁-C₄ alkyl), --NH(C₁-c₄ alkyl), --NH₂, --CO₂H, --C(O)OC₁-c₄ alkyl, --CON(C₁-c₄ alkyl)(C₁-C₄ alkyl), --CONH(C₁-c₄ alkyl), and --CONH₂, and where each of said aryl, heteroaryl, cycloalkyl, or heterocycloalkyl is optionally unsubstituted or substituted with one or more substituents independently selected from C₁-c₄ alkyl, C₁-c₄ haloalkyl, halogen, --OR^a, --SR^a, --NR^{aRa}, --CON(C₁-c₄ alkyl)(C₁-c₄ alkyl), --CONH(C₁-c₄ alkyl), --CONH₂, nitro and cyano; R³ is H, halogen or --C(O)OH; R⁴ is H, halogen, or C₁-c₄ alkyl; R⁵ is H, halogen, C₁-c₄ alkyl, or --OR^a; R⁶ is H, halogen, or --OR^a; R⁷ is hydrogen, C₁-c₆ alkyl, C₂-c₆ alkenyl, C₂-c₆ alkynyl, aryl, heteroaryl, nitro, cyano, halogen, --C(O)OR^a, --C(O)C₁-c₆ alkyl, --C(O)NR^{aRd}, --OR^b, --NR^{aRd}, --N(R^a)C(O)R^d, OC(O)NR^{aRd}, or --N(R^a)C(O)NR^{aRd}, where said alkyl, alkenyl or alkynyl is unsubstituted or substituted with one or more substituents independently selected from halogen, --OR^a, --SR^a, --NR^{aRa}, cyano, nitro, --CO₂H, --C(O)OC₁-c₄ alkyl, --CON(C₁-c₄ alkyl)(C₁-c₄ alkyl), --CONH(C₁-c₄ alkyl), --CONH₂, aryl, and heteroaryl, and where said aryl or heteroaryl is unsubstituted or substituted with one or more substituents independently selected from C₁-c₆ alkyl, C₁-c₆ haloalkyl, halogen, --OR^a, --SR^a, --NR^{aRa}, cyano and nitro; R⁸ is hydrogen or halogen; or R¹ and R² or R⁵ and R⁶ or R⁶ and R⁷ or R⁷ and R⁸ taken together are alkylenedioxy; W is hydrogen, --C(O)OR^a, C₃-c₈ alkyl, C₃-c₆ alkenyl, C₃-c₆ alkynyl, --(C₁-c₄ alkyl)-(C₃-c₆ cycloalkyl), --(C₁-c₄ alkyl)-heterocycloalkyl, --(C₁-c₄ alkyl)-aryl, or --(C₁-c₄ alkyl)-heteroaryl, where the C₁-c₈ alkyl, C₂-c₆ alkenyl or C₂-c₆ alkynyl is unsubstituted or substituted with one or more substituents independently selected from halogen, cyano, --OR^a, --SR^a, --S(O)C₁-c₄ alkyl, --S(O)₂C₁-c₄ alkyl, and where the cycloalkyl,

alkyl)-(C₃₋₆ cycloalkyl), --(C₁₋₄ alkyl)-heterocycloalkyl, --(C₁₋₄ alkyl)-aryl, or --(C₁₋₄ alkyl)-heteroaryl is unsubstituted or substituted with one or more substituents independently selected from C₁₋₄ alkyl, C₁₋₄ haloalkyl, halogen, nitro, cyano, --OR_a, --NR_aR_a; X is O; Y is OH; and Z is hydrogen or methyl; each R_a is independently H or C₁₋₄ alkyl; each R_b is independently H or C₁₋₄ alkyl, where the alkyl is optionally unsubstituted or substituted by one or more substituents independently selected from the group consisting of halogen, cyano, --OC₁₋₄ alkyl, --OH, --N(C₁₋₄ alkyl)(C₁₋₄ alkyl), --NH(C₁₋₄ alkyl), --NH₂, --CO₂H, --C(O)OC₁₋₄ alkyl, --CON(C₁₋₄ alkyl)(C₁₋₄ alkyl), --CONH(C₁₋₄ alkyl), --CONH₂, aryl, heteroaryl, heterocycloalkyl, --C(O)aryl, --C(O)heterocycloalkyl, and --C(O)heteroaryl, where said aryl, heteroaryl, heterocycloalkyl, --C(O)aryl, --C(O)heterocycloalkyl, or --C(O)heteroaryl is unsubstituted or substituted with one or more substituents independently selected from C₁₋₄ alkyl, C₁₋₄ haloalkyl, halogen, --OR_a, --SR_a, --NR_aR_a, cyano and nitro; each R_c is independently C₁₋₄ alkyl, optionally unsubstituted or substituted by one or more substituents independently selected from the group consisting of halogen, cyano, --OC₁₋₄ alkyl, --OH, --N(C₁₋₄ alkyl)(C₁₋₄ alkyl), --NH(C₁₋₄ alkyl), --NH₂, --CO₂H, --C(O)OC₁₋₄ alkyl, --CON(C₁₋₄ alkyl)(C₁₋₄ alkyl), --CONH(C₁₋₄ alkyl), --CONH₂, aryl and heteroaryl, and where said aryl or heteroaryl is unsubstituted or substituted with one or more substituents independently selected from C₁₋₄ alkyl, C₁₋₄ haloalkyl, halogen, --OR_a, --SR₁, --NR_aR_a, cyano and nitro; each R_d is independently H or C₁₋₄ alkyl, where the alkyl is optionally substituted by one or more substituents independently selected from the group consisting of halogen, cyano, --OC₁₋₄ alkyl, --OH, --N(C₁₋₄ alkyl)(C₁₋₄ alkyl), --NH(C₁₋₄ alkyl), --NH₂, --CO₂H, --C(O)OC₁₋₄ alkyl, --CON(C₁₋₄ alkyl)(C₁₋₄ alkyl), --CONH(C₁₋₄ alkyl), --CONH₂, --C(O)C₁₋₄ alkyl, --C(O)aryl, --C(O)heteroaryl, cycloalkyl, heterocycloalkyl, aryl and heteroaryl, and where said aryl or heteroaryl is unsubstituted or substituted with one or more substituents independently selected from C₁₋₄ alkyl, C₁₋₄ haloalkyl, halogen, --OR_b, --SR_a, --NR_aR_a, cyano and nitro; or, when present in any NR_aR_b or NR_aR_d, each R_a and R_b or each R_a and R_d, independently, taken together with the nitrogen atom to which they are attached form a 5- or 6-membered heterocycloalkyl ring, which optionally contains one or more heteroatoms selected from oxygen or nitrogen and which is unsubstituted or substituted with one or more substituents selected from the group halogen, cyano, --OC₁₋₄ alkyl, --OH, --N(C₁₋₄ alkyl)(C₁₋₄ alkyl), --NH(C₁₋₄ alkyl), --NH₂, --CO₂H, --C(O)OC₁₋₄ alkyl, --C(O)C₁₋₄ alkyl, --CON(C₁₋₄ alkyl)(C₁₋₄ alkyl), --CONH(C₁₋₄ alkyl), --CONH₂, --C(O)C₁₋₄ alkyl; provided that when Z, R¹, R², R³, R⁴, R⁵, R⁶, R⁷ and R⁸ are hydrogen: W is not hydrogen, --CH₃, --C₂H₅, --IC₃H₇, --nC₄H₉, --nC₅H₁₁, --nC₆H₁₃, --nC₇H₁₅, --(CH₂)CH(CH₃)₂, --(CH₂)₂CH(CH₃)₂, --CH₂CH.dbd.CH₂, --CH₂CH.dbd.CH(CH₃), --(CH₂)₃CN, --(CH₂)₄CN, --(CH₂)phenyl, --(CH₂)pyridin-2-yl, or --(CH₂)₂₀H₃; or a tautomer thereof, or a pharmaceutically acceptable salt or solvate thereof.

3. The compound according to claim 1, wherein: R¹ is H, phenyl, --CH₃, F, Cl, Br, --OH, --C(O)OH, or --C(O)NHCH₃; R² is H, F, Cl, Br, I, --OH, --OCH₃, --CH₃, --CH₂(4-OCH₃-phenyl), --CH.dbd.CHC(O)NH₂, --NO₂, --NH₂, --NHCH₃, --N(CH₃)₂, --CONHCH₃, --CON(CH₃)₂, --CO₂H, --CO₂CH₂CH₃, --O(CH₂)₂CH(CH₃)₂, --O(CH₂)₃CN, --OCH₂CN, --O(CH₂)₂₀CH₃, --O(CH₂)₂₀H, --OCH₂CH(OH)CH₂CH₃, --O(CH₂)₂N(CH₃)₂, --OCH₂phenyl, --OCH₂CONH₂, --O(6-Br-pyridin-2-yl), --O(6OCH₃-pyridin-2-yl), --OSi(CH₃)₂(tBu), --NHCH₂CO₂H, --NHCH₂CO₂CH₂CH₃, --NHCH₂-2-furyl, --NH(CH₂)₂₀H, --NHCH₂CN, --NHCH₂C(O)NH₂, --NHC(O)CH₃, --NHC(O)CH₂CH(CH₃)₂, --NHC(O)CH₂N(CH₃)₂, --NHC(O)phenyl, --NHC(O)(3-CH₃O-

--NHC(O)(3-CF₃-phenyl), --NHC(O)(3-F-phenyl), --NHC(O)(3-pyridyl),
 --NHC(O)(2-furyl), --NHC(O)(2-thienyl), --NHC(O)(4-OCH₃-phenyl),
 --NHC(O)(cyclopentyl); R³ is H, F, Cl, Br, or --CO₂H;
 R⁴ is H, Br or --(CH₂)₂CH(CH₃)₂; R⁵ is H,
 --CH₃, --OCH₃ or --OH; R⁶ is H, Br, --OH, or
 --OCH₃; R⁷ is H, --CH₃, --OH, --OCH₃, phenyl, F,
 Cl, Br, I, NO₂, --NH₂, --N(CH₃)₂, --NHCH₂CN,
 --CN, --CH₂NH₂, --CH₂CH₂C(O)NH₂,
 --CH.dbd.CHC(O)NH₂, --(CH₂)₂CH(CH₃)OCH₃, --CHO,
 --C(O)CH₃, --CO₂CH₃, --CO₂H, --C(O)NH₂,
 --C(O)NHCH₃, --C(O)N(CH₃)₂, --OCH₂CO₂CH₃,
 --OCH₂CO₂H, --OCH₂CH(NH₂)CH₂CH₃,
 --O(CH₂)₂N(CH₃)₂, --OCH₂CN,
 --O(CH₂)₂NH₂, --OCH₂C(O)NH₂,
 --OCH₂CONHCH₃, --OCH₂CON(CH₃)₂,
 --OCH(CH₃)C(O)NH₂, --OCH₂-tetrazol-5-yl,
 --OCH₂C(O)(3-pyridyl), --OCH₂C(O)(N-pyrrolidinyl),
 --OCH₂C(O)(N-piperazinyl), --OCH₂C(O)(N-morpholinyl),
 --OCH₂(5-methyl-1,3,4-oxadiazol-2-yl), --C(O)NH(CH₂)₃(N-
 imidazolyl), --C(O)NHCH₂CH(OCH₃)₂, --C(O)(4-
 acetylpiperizin-1-yl), --C(O)NHCH₂(2-tetrahydrofuryl),
 --C(O)NHCH₂phenyl, --C(O)NH(CH₂)₃N(CH₂CH₃)₂,
 --C(O)(N-pyrrolidinyl), --C(O)NH(CH₂)₂(4-OCH₃phenyl),
 or --NHCH₂phenyl; R⁸ is H; or R¹ and R² taken
 together are methylenedioxy; W is selected from the group consisting of
 --(CH₂)₁₋₃-phenyl, --CH₂-(2-CN-phenyl),
 --(CH₂)₁₋₂-cyclopropyl, --CH₂-(2-CH₃-cycloprop-1-yl),
 --CH₂-cyclobutyl, --(CH₂)-cyclopentyl, --(CH₂)-
 cyclohexyl, --CH₂-(2-tetrahydrofuryl), --CH₂-(3-
 tetrahydrofuryl), --CH₂-3-pyridyl, --CH₂-(6-NH₂-3-
 pyridyl), --CH₂-(4-pyridyl), --CH₂-(2-NH₂-4-pyridyl),
 --CH₂-(2-CH₃ pyridyl), --CH₂-(4-bromophenyl),
 --CH₂-(3-bromophenyl), --CH₂-(3-NO₂-phenyl),
 --CH₂-(3-furyl), --(CH₂)₂-(2-thienyl),
 --CH₂-(3-thienyl), --(CH₂)₂CH(CH₃)₂,
 --(CH₂)₂C(CH₃)₃, --CH₂CH(CH₃)CH.sub
 .2CH₃, --(CH₂)₂CH(CH₃)(CF₃),
 --(CH₂)₂CH₂, --CH₂CH.dbd.CH₂, --
 (CH₂)₂CHBr(CH₃), --(CH₂)CH.dbd.C(CH₃)₂,
 --(CH₂)₃CF₃, --(CH₂)₃CN, --(CH₂)₃-
 4OH, --(CH₂)₂CH(CH₃)OCH₃, --CH₂CH₂.tbd.CH,
 --(CH₂)₃C.dbd.CH, --CO₂CH₂CH₃,
 --(CH₂)₂CH(CH₃)CH₂CH₃, --
 (CH₂)₂SCH₃, (CH₂)₃SCH₃,
 --(CH₂)₂S(O)CH₃, --CH₂)₂S(O)CH₃; X
 is O; Y is OH; and Z is hydrogen or methyl; provided that when Z,
 R¹, R², R³, R⁴, R⁵, R⁶, R⁷ and
 R⁸ are hydrogen: W is not --(CH₂)₂CH(CH₃)₂,
 --CH₂CH.dbd.CH₂, --CH₂CH.dbd.CH(CH₃),
 --(CH₂)₃CN, or --(CH₂)phenyl; or a tautomer thereof, or
 a pharmaceutically acceptable salt or solvate thereof.

4. The compound according to claim 1, wherein: R¹ and R³ are
 each independently H or F; R² is hydrogen halogen, --OR^{b'},
 --NHR^{b'}, NO₂, where R^{b'} is H or C₁₋₂ alkyl,
 where the C₁₋₂ alkyl is optionally unsubstituted or
 substituted by a substituent selected from the group consisting of
 cyano, --OH, --CO₂H, --CONH₂, --C(O)OC₁₋₂ alkyl,
 --CONH(C₁₋₂ alkyl), and unsubstituted monocyclic heteroaryl;
 R⁴, R⁶ and R⁸ are each H; R⁵ is H or --OH; R⁷
 is hydrogen, halogen, C₁₋₂ alkyl, C₂ alkenyl,
 --C(O)OR^{a'}; --C(O)R^{a'}; --OR^{b''}, NR^{a'}R^{d'};
 --C(O)NR^{a'}R^{d'}, where said alkyl or alkenyl is unsubstituted or
 substituted a substituent selected from --NH₂ and --CONH₂,
 R^{4'} is H or methyl, R^{b''} is H or C₁₋₄ alkyl, where
 the C₁₋₄ alkyl is optionally unsubstituted or substituted by
 a substituent selected from the group consisting of cyano, --NH₂,
 --CO₂H, --CONH₂, --C(O)OC₁₋₂ alkyl,
 --CON(C₁₋₄ alkyl)(C₁₋₄ alkyl),
 --CONH(C₁₋₄ alkyl), monocyclic heteroaryl, --C(O)monocyclic
 heterocycloalkyl, and --C(O)-monocyclic heteroaryl, where said
 heteroaryl, --C(O)heterocycloalkyl, or --C(O)heteroaryl are
 unsubstituted or substituted one or more of C₁₋₄ alkyl,
 halogen, cyano, --OH, --NH₂, and --CONH₂, R^{d'} is H or
 C₁₋₂ alkyl, where the C₁₋₂ alkyl is unsubstituted
 or substituted by a substituent selected from the group consisting of
 cyano and unsubstituted aryl, or R^{a'} and R^{d'} taken together
 with the nitrogen atom to which they are attached form a 5- or 6membered

heteroatom and which is unsubstituted or substituted with --(O)C₁₋₂ alkyl; or R¹ and R² taken together are alkylenedioxy; W is C₄₋₆ alkyl, C₄alkenyl, C₄alkynyl, --(C₁₋₂ alkyl)-(C₃₋₆ cycloalkyl), --(C₁₋₄ alkyl)-heterocycloalkyl, --(C₁₋₄ alkyl)-aryl, or --(C₁ alkyl)-heteroaryl, where the C₄₋₆ alkyl, C₄ alkenyl or C₄ alkynyl is unsubstituted or substituted with one or more substituents independently selected from halogen, --OH, --OCH₃, --SCH₃, and where the cycloalkyl, heterocycloalkyl, aryl or heteroaryl moiety of the --(C₁₋₄ alkyl)-(C₃₋₆ cycloalkyl), --(C₁₋₄ alkyl)-heterocycloalkyl, --(C₁₋₄ alkyl)-aryl, or --(C₁₋₄ alkyl)-heteroaryl is unsubstituted or substituted with one or more substituents independently selected from --CH₃, halogen, nitro, cyano, --OR^a, --NR^aR^a; X is O; Y is OH; and Z is hydrogen; provided that when R¹, R², R³, R⁴, R⁵, R⁶, R⁷ and R⁸ are hydrogen: W is not -nC₄H₉, -nC₅H₁₁, -nC₆H₁₃, -nC₇H₁₅, --(CH₂)CH(CH₃)₂, --(CH₂)₂CH(CH₃)₂, --CH₂CH.dbd.CH(CH₃), --(CH₂)phenyl, or --(CH₂)pyridin-2-yl; or a tautomer thereof, or a pharmaceutically acceptable salt or solvate thereof.

5. A compound according to any one of claims 1-4, having the formula: ##STR13## or a tautomer thereof, or a pharmaceutically acceptable salt or solvate thereof.

6. A compound according to any one of claims 14, having the formula: ##STR14## or a tautomer thereof, or a pharmaceutically acceptable salt or solvate thereof.

7. A compound selected from the group consisting of:
1-cyclopropylmethyl-3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1H-quinolin-2-one, 3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-6-nitro-1H-quinolin-2-one, 6-chloro-3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-1H-quinolin-2-one, 6-bromo-3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-1H-quinolin-2-one, 3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-3-methylbutyl)-1H-quinolin-2-one, 3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-6-methoxy-1-(3-methylbutyl)-H-quinolin-2-one, 1-but-3-enyl-3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1H-quinolin-2-one, 1-(3-bromobutyl)-3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-1H-quinolin-2-one, N-[3-(1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-2-oxo-1,2-dihydro-quinolin-6-yl]A4-fluorobenzamide, 1-cyclohexylmethyl-3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1H-quinolin-2-one, 3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbut-2-enyl)-1H-quinolin-2-one, 3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-6-methyl-1-(3-methylbutyl)-1H-quinolin-2-one, 3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-6-fluoro-4-hydroxy-1-(3-methylbutyl)-1H-quinolin-2-one, 3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-5-fluoro-4-hydroxy-1-(3-methylbutyl)-1H-quinolin-2-one, 6,7-difluoro-3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-1H-quinolin-2-one, 3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-7-fluoro-4-hydroxy-1-(3-methylbutyl)-1H-quinolin-2-one, 3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(4,4,4-trifluorobutyl)-1H-quinolin-2-one, 6-amino-3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl) hydroxy-1-(3-methylbutyl)-1H-quinolin-2-one, 3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(4-hydroxybutyl)-1H-quinolin-2-one, 3-(1,1-dioxo-1,2-dihydro-7-nitrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-1H-quinolin-2-one, 3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-pent-4ynyl-1H-quinolin-2-one, 3-(7-bromo-1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-1H-quinolin-2-one, 3-(7-amino-1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-1H-quinolin-2-one, 3-(7-cyano-1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-1H-quinolin-2-one, 8-bromo-3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1H-quinolin-2-one, 6-amino-3-(7-amino-1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-1H-quinolin-2-one, 1-cyclopentylmethyl-3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1H-quinolin-2-one, 3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-5-methyl-1-(3-methylbutyl)-1H-quinolin-2-one, 5-chloro-3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-1H-quinolin-2-one, 5-bromo-3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-

4-[3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-4-methoxy-2-oxo-2H-quinolin-1-yl]butyronitrile, 1-but-3-ynyl-3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1H-quinolin-2-one, 1-(3,3-dimethylbutyl)-3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1H-quinolin-2-one, 1-(2-cyclopropylethyl)-3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1H-quinolin-2-one, furan-2-carboxylic acid, [3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-2-oxo-1,2-dihydroquinolin-6-yl]amide, 3-cyano-N-[3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-2-oxo-1,2-dihydroquinolin-6-yl]benzamide, cyclopentanecarboxylic acid [3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-2-oxo-1,2-dihydroquinolin-6-yl]amide, N-[3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-2-oxo-1,2-dihydroquinolin-6-yl]-3-methoxybenzamide, N-[3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-2-oxo-1,2-dihydroquinolin-6-yl]-benzamide, N-[3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-2-oxo-1,2-dihydroquinolin-6-yl]4-nitrobenzamide, 3-[4-hydroxy-1-(3-methylbutyl)-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazine-7-carboxylic acid amide, 3-(6-bromo-1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-1H-quinolin-2-one, N-[3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-2-oxo-1,2-dihydroquinolin-6-yl]-3-methylbutyramide, [3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-2-oxo-1,2-dihydroquinolin-6-ylamino]acetic acid ethyl ester, 4-hydroxy-1-(3-methylbutyl)-3-(5-methyl-1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-1H-quinolin-2-one, 2-dimethylamino-N-[3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-2-oxo-1,2-dihydroquinolin-6-yl]acetamide, 3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(2-methylsulfanylethyl)-1H-quinolin-2-one, 3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylsulfanylpropyl)-1H-quinolin-2-one, 3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-1-furan-3-ylmethyl-4-hydroxy-1H-quinolin-2-one, 3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(2-thiophen-2-yl-ethyl)-1-quinolin-2-one, 3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(2-thiophen-3-yl-ethyl)-1H-quinolin-2-one, 3-(7-chloro-1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-1H-quinolin-2-one, 3-[4-hydroxy-1-(3-methylbutyl)-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazine-7-carboxylic acid, methyl ester, 3-[4-hydroxy-1-(3-methylbutyl)-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazine-7-carboxylic acid, 6-(tert-butyl)dimethylsilyloxy)-3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-1H-quinolin-2-one, 3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4,6-dihydroxy-1-(3-methylbutyl)-1H-quinolin-2-one, 3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(4,4,4-trifluoro-3-methylbutyl)-1H-quinolin-2-one, 4-hydroxy-3-(7-methoxy-1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-1-(3-methylbutyl)-1H-quinolin-2-one, [3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-2-oxo-1,2-dihydroquinolin-6-ylamino]-acetic acid, 3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-5-phenyl-1H-quinolin-2-one, 4-hydroxy-3-(7-hydroxy-1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-1-(3-methylbutyl)-1H-quinolin-2-one, 3-[4-hydroxy-1-(3-methylbutyl)-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-7-yloxy)-acetic acid, methyl ester, [3-[4-hydroxy-1-(3-methylbutyl)-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-7-yloxy)-acetic acid, 1-(2-cyclopropylethyl)-3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-6-nitro-1H-quinolin-2-one, 3-[4-hydroxy-1-(3-methylbutyl)-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazine-7-carboxylic acid dimethylamide, 6-amino-1-(2-cyclopropylethyl)-3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1H-quinolin-2-one, 3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(2-methanesulfinylethyl)-1H-quinolin-2-one, 3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(2-methanesulfonylethyl)-1H-quinolin-2-one, (2-cyclopropylethyl)-6-dimethylamino-1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1H-quinolin-2-one, 1-(2-cyclopropylethyl)-3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-6-methylamino-1H-quinolin-2-one, 1-(2-cyclopropylethyl)-3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-6-fluoro-4-hydroxy-1H-quinolin-2-one, 1-(2-cyclopropylethyl)-6-(2-dimethylaminoethoxy)-3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1H-quinolin-2-one, 3-[7-(2-dimethylaminoethoxy)-1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-1H-quinolin-2-one, 3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-2-oxo-1,2-dihydroquinoline-6-carboxylic acid methylamide, 1-(2-cyclopropylethyl)-4-hydroxy-3-(7-methoxy-1,1-dioxo-

1-(2-cyclopropylethyl)-3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-6-[furan-2-ylmethylamino]-4-hydroxy-1H-quinolin-2-one, 3-[1-(2-cyclopropylethyl)-4-hydroxy-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazine-7-carboxylic acid dimethylamide, 1-(2-cyclopropylethyl)-4-hydroxy-3-(7-iodo-1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-1H-quinolin-2-one, 1-(2-cyclopropylethyl)-3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-6-(2-hydroxyethoxy)-1H-quinolin-2-one, 3-[1-(2-cyclopropylethyl)-4-hydroxy-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazine-7-carboxylic acid methylamide, 3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-2-oxo-1,2-dihydroquinoline-5-carboxylic acid methylamide, 1-(3,3-dimethylbutyl)-3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-6-nitro-1H-quinolin-2-one, 3-[6-amino-4-hydroxy-1-(3-methylbutyl)-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazine-7-carboxylic acid amide, 6-amino-1-(3,3-dimethylbutyl)-3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1H-quinolin-2-one, 3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-2-oxo-2H-quinoline-1-carboxylic acid ethyl ester, 1-(3,3-dimethylbutyl)-3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-6-(2-hydroxyethylamino)-1H-quinolin-2-one, 6-benzyloxy-3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-1H-quinolin-2-one, {3-[4-hydroxy-1-(3-methylbutyl)-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-7-yloxy}acetonitrile, 3-[7-(2-aminoethoxy)-1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl]-4-hydroxy-1-(3-methylbutyl)-1H-quinolin-2-one, 2-{3-[4-hydroxy-1-(3-methylbutyl)-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-7-yloxy}acetamide, 3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(tetrahydrofuran-3-ylmethyl)-1H-quinolin-2-one, 3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(tetrahydrofuran-2-ylmethyl)-1H-quinolin-2-one, 3-7-fluoro-1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl+hydroxy-1-(3-methylbutyl)-1H-quinolin-2-one, 4-[3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-2-oxo-1,2-dihydroquinolin-6-yloxy]butyronitrile, 3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-6-(2-methoxyethoxy)-1-(3-methylbutyl)-1H-quinolin-2-one, 3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-2-oxo-1,2-dihydroquinoline-6-carboxylic acid, 3-[1-(2-cyclopropylethyl)-4-hydroxy-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazine-7-carboxylic acid (3-diethylaminopropyl)amide, 3-[1-(2-cyclopropylethyl)-4-hydroxy-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazine-7-carboxylic acid benzylamide, 1-2-cyclopropylethyl-3-[1,1-dioxo-7-(1-pyrrolidin-1-yl)-methanoyl]-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl+4-hydroxy-1H-quinolin-2-one, 3-(7-[1-(4-acetylpiperazin-1-yl)-methanoyl]-1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-1-(2-cyclopropylethyl)-4-hydroxy-1H-quinolin-2-one, 3-[1-(2-cyclopropylethyl)-4-hydroxy-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazine-7-carboxylic acid (tetrahydrofuran-2-ylmethyl)-amide, 3-[1-(2-cyclopropylethyl)-4-hydroxy-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazine-7-carboxylic acid (2,2-dimethoxyethyl)amide, 3-[1-(2-cyclopropylethyl)-4-hydroxy-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazine-7-carboxylic acid (3-imidazol-1-ylpropyl)amide, 4-hydroxy-3-(5-methoxy-1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-1-(3-methylbutyl)-1H-quinolin-2-one, 4-hydroxy-3-(5-hydroxy-1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-1-(3-methylbutyl)-1H-quinolin-2-one, 3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-1-(3-methylbutyl)-1H-quinolin-2-one, (E)-3-(3-[4-hydroxy-1-(3-methylbutyl)-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-7-yl)acrylamide, 3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4,5,6-trihydroxy-1-(3-methylbutyl)-1H-quinolin-2-one, 3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methoxybutyl)-1H-quinolin-2-one, 3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1 pyridin-3-ylmethyl)-1H-quinolin-2-one, 3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(pyridin-4-ylmethyl)-1H-quinolin-2-one, 3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-(5,6-methylenedioxy)-1H-quinolin-2-one, 3-(1,1-dioxo-7-(2-oxo-2-pyridin-3-ylethoxy)-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-1H-quinolin-2-one, 3-(1,1-dioxo-4-methyl-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-1H-quinolin-2-one, 3-(1,1-dioxo-7-methyl-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)+hydroxy-1-(3-methylbutyl)-1H-quinolin-2-one, 3-(1,1-dioxo-1,4-

quinolin-2-one, 3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-6-(2-hydroxybutoxy)-1-(3-methylbutyl)-1H-quinolin-2-one, 3-(7-dimethylamino-1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-1H-quinolin-2-one, 3-{7-[(2R)-aminobutoxy]-1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-1H-quinolin-2-one, 3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(4-nitrobenzyl)-1H-quinolin-2-one, 1-(6-aminopyridin-3-ylmethyl)-3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1H-quinolin-2-one, 1-(4-bromobenzyl)-3-(1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1H-quinolin-2-one, 1-(3-bromobenzyl)-3-(1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1H-quinolin-2-one, 1-(2-cyclopropylethyl)-6-fluoro-4-hydroxy-3-(7-methoxy-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-1H-quinolin-2-one, 3-(7-benzylamino-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-1H-quinolin-2-one, 3-{3-[1-(2-cyclopropylethyl)-6-fluoro-4-hydroxy-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-7-yl}propionamide, 1-(2-cyclopropylethyl)-6-fluoro-4-hydroxy-3-(7-hydroxy-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-1H-quinolin-2-one, {3-[1-(2-cyclopropylethyl)-6-fluoro-4-hydroxy-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-7-yloxy}acetamide, 2-{3-[1-(2-cyclopropylethyl)-6-fluoro-4-hydroxy-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-7-yloxy}acetamide, 3-(1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(2-methylcyclopropylmethyl)-1H-quinolin-2-one, 1-(2-aminopyridin-4-ylmethyl)-3-(1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1H-quinolin-2-one, 6-fluoro-4-hydroxy-3-(7-hydroxy-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-1-(3-methylbutyl)-1H-quinolin-2-one, {3-[6-fluoro-4-hydroxy-1-(3-methylbutyl)-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-7-yloxy}acetonitrile, 4,6-dihydroxy-3-(7-hydroxy-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-1-(3-methyl-butyl)-1H-quinolin-2-one, {3-(7-cyanomethoxy-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methyl-butyl)-2-oxo-1,2-dihydroquinolin-6-yloxy}-acetonitrile, 2-{3-[1-(2-cyclopropyl-ethyl)-6-fluoro-4-hydroxy-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-7-yloxy}-propionamide, 1-(2-cyclopropyl-ethyl)-3-[1,1-dioxo-7-(tetrazol-5-yloxy)-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl]-6-fluoro-4-hydroxy-1H-quinolin-2-one, 2-{3-[6-fluoro-4-hydroxy-1-(3-methyl-butyl)-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-7-yloxy}-acetamide, 2-{3-(7-carbamoylmethoxy-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methyl-butyl)-2-oxo-1,2-dihydroquinolin-6-yloxy}-acetamide, 2-{3-(7-carbamoylmethoxy-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-1-(2-cyclopropyl-ethyl)-4-hydroxy-2-oxo-1,2-dihydroquinolin-6-yloxy}-acetamide, {3-(7-cyanomethoxy-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methyl-butyl)-2-oxo-1,2-dihydroquinolin-6-ylamino}-acetonitrile, 2-{3-[1-(2-cyclopropyl-ethyl)-6-fluoro-4-hydroxy-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-7-yloxy}-2-methyl-propionamide, 3-(1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-nitrobenzyl)-1H-quinolin-2-one, 3-(1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(2-methylpyridin-4-ylmethyl)-1H-quinolin-2-one, {3-[7-(cyanomethyl-amino)-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl]4-hydroxy-1-(3-methyl-butyl)-2-oxo-1,2-dihydroquinolin-6-ylamino}-acetonitrile, 3-(7-amino-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-1-(2-cyclopropylethyl)-6-fluoro-4-hydroxy-1H-quinolin-2-one, {3-[1,12-cyclopropylethyl)-6-fluoro-4-hydroxy-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-7-yloxy}acetic acid, 2-{3-(7-carbamoylmethoxy-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-2-oxo-1,2-dihydroquinolin-6-ylamino}acetamide, 6-fluoro-4-hydroxy-3-(7-hydroxy-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-1-pyridin-4-ylmethyl-1H-quinolin-2-one, 1-(2-cyclopropylethyl)-6-fluoro-4-hydroxy-3-[7-(5-methyl-1,3,4-oxadiazol-2-ylmethoxy)-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl]-1H-quinolin-2-one, 6-chloro-4-hydroxy-3-(7-hydroxy-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-1-(3-methylbutyl)-1H-quinolin-2-one, {3-[6-chloro-4-hydroxy-1-(3-methylbutyl)-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-7-yloxy}acetonitrile, 2-{3-[6-chloro-4-hydroxy-1-(3-methyl-butyl)-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-7-yloxy}acetamide, 3-(7-amino-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-6-fluoro-4-hydroxy-1-(3-methylbutyl)-1H-quinolin-2-one, {3-[6-amino-4-hydroxy-1-(3-methylbutyl)-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-7-yloxy}acetonitrile, 2-{3-[1-(2-cyclopropylethyl)-6-fluoro-4-hydroxy-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-7-yloxy}-N,N-dimethylacetamide, 2-{3-[1-(2-cyclopropylethyl)-6-fluoro-4-hydroxy-2-

benzo[1,2,4]thiadiazin-7-yloxy)-N-methylacetamide, {3-[4-hydroxy-6-(2-hydroxyethylamino)-1-(3-methylbutyl)-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-7-yloxy}acetoneitrile, 1-(2-cyclopropylethyl)-3-[1,1-dioxo-7-(2-oxo-2-pyrrolidin-1-yl-ethoxy)-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl]-6-fluoro-4-hydroxy-1H-quinolin-2-one, 1-(2-cyclopropylethyl)-6-fluoro-4-hydroxy-3-[7-(2-morpholin-4-yl-2-oxo-ethoxy)-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl]-1H-quinolin-2-one, 2-[3-[6-amino-4-hydroxy-1-(3-methylbutyl)-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-7-yloxy}acetamide, 3-(1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(2-methyl-thiazol-4-ylmethyl)-1H-quinolin-2-one, 2-[3-[6-chloro-1-(2-cyclopropylethyl)-4-hydroxy-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-7-yloxy}acetamide, 1-(2-cyclopropylethyl)-3-[1,1-dioxo-7-(2-oxo-2-piperazin-1-yl-ethoxy)-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl]-6-fluoro-4-hydroxy-1H-quinolin-2-one, 1-cyclobutylmethyl-6-fluoro-4-hydroxy-3-(7-hydroxy-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-1H-quinolin-2-one, [3-(1-cyclobutylmethyl-6-fluoro-4-hydroxy-2-oxo-1,2-dihydroquinolin-3-yl)-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-7-yloxy}acetoneitrile, 2-[3-(1-cyclobutylmethyl-6-fluoro-4-hydroxy-2-oxo-1,2-dihydroquinolin-3-yl)-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-7-yloxy}acetamide, {3-[1-(3,3-dimethylbutyl)-6-fluoro-4-hydroxy-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydro-benzo[1,2,4]thiadiazin-7-yloxy}acetoneitrile, 1-cyclobutylmethyl-6-fluoro-4-hydroxy-3-(7-methoxy-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-1H-quinolin-2-one, 1-(3,3-dimethylbutyl)-6-fluoro-4-hydroxy-3-(7-methoxy-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-1H-quinolin-2-one, 3-(7-acetyl-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-1H-quinolin-2-one, (S)-2-(3-[1-(2-cyclopropylethyl)-6-fluoro-4-hydroxy-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-7-yloxy}propionamide, (R)-2-(3-[1-(2-cyclopropylethyl)-6-fluoro-4-hydroxy-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-7-yloxy}propionamide, {3-[1-(3,3-dimethylbutyl)-6-fluoro-4-hydroxy-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydro-benzo[1,2,4]thiadiazin-7-yloxy}acetamide, 3-[3-(1-(2-cyclopropylethyl)-6-fluoro-4-hydroxy-2-oxo-1,2-dihydroquinolin-3-yl)-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-7-yl]-3-oxopropionamide, 2-[3-[4-hydroxy-6-(2-hydroxyethylamino)-1-(3-methylbutyl)-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-7-yloxy}acetamide, 2-(3-[1-(2-cyclopropylethyl)-4-hydroxy-6-(2-hydroxyethylamino)-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-7-yloxy}acetamide, 2-[3-[1-(2-cyclopropylethyl)-6-fluoro-4-hydroxy-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-7-ylamino}acetamide, and 2-[3-[1-(2-cyclopropylethyl)-6-fluoro-4-hydroxy-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-7-yloxy]-2-methylpropionic acid, or a tautomer thereof, or a pharmaceutically acceptable salt or solvate thereof.

8. A pharmaceutically acceptable salt of the compound according to claim 7, or tautomer thereof, wherein said pharmaceutically acceptable salt is a sodium salt or a potassium salt.

9. The compound according to claim 7, selected from the group consisting of: 1-(2-cyclopropylethyl)-6-fluoro-4-hydroxy-3-(7-hydroxy-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-1H-quinolin-2-one, {3-[1-(2-cyclopropylethyl)-6-fluoro-4-hydroxy-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-7-yloxy}acetoneitrile, 3-[4-hydroxy-1-(3-methylbutyl)-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazine-7-carboxylic acid amide, 2-[3-[1-(2-cyclopropylethyl)-6-fluoro-4-hydroxy-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-7-yloxy}acetamide, 2-[3-(7-carbamoylmethoxy-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-2-oxo-1,2-dihydroquinolin-6-yloxy]-acetamide, 1-(2-cyclopropylethyl)-3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-6-fluoro-4-hydroxy-1H-quinolin-2-one, 2-[3-(4-hydroxy-1-(3-methylbutyl)-2-oxo-1,2-dihydroquinolin-3-yl)-1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-7-yloxy}acetamide, 3-[3-[1-(2-cyclopropylethyl)-6-fluoro-4-hydroxy-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-7-yl]propionamide, {3-[6-fluoro-4-hydroxy-1-(3-methylbutyl)-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-7-yloxy}acetoneitrile, 2-[3-(7-carbamoylmethoxy-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-1-(2-cyclopropyl-ethyl)-4-hydroxy-2-oxo-1,2-dihydroquinolin-6-yloxy]-acetamide, 1-(2-cyclopropyl-ethyl)-3-[1,1-dioxo-7-(tetrazol-5-ylmethoxy)-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl]-6-fluoro-4-hydroxy-1H-quinolin-2-one, 2-[3-[1-(2-cyclopropyl-ethyl)-6-fluoro-4-hydroxy-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-7-yloxy]-propionamide, 6-amino-3-(7-amino-1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-

dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-7-yloxy)acetonitrile, (E)-3-{3-[4-hydroxy-1-(3-methylbutyl)-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-7-yl}acrylamide, 6-fluoro-4-hydroxy-3-(7-hydroxy-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-1-(3-methylbutyl)-1H-quinolin-2-one, 2-{3-[1-(2-cyclopropyl-ethyl)-6-fluoro-4-hydroxy-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-7-yloxy}-2-methylpropionamide, {3-(7-cyanomethoxy-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-2-oxo-1,2-dihydroquinolin-6-yloxy}acetonitrile, {3-[1-(2-cyclopropylethyl)-6-fluoro-4-hydroxy-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-7-yloxy}acetic acid, 2-{3-(7-carbamoylmethoxy-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-2-oxo-1,2-dihydroquinolin-6-ylamino}acetamide, 6-fluoro-4-hydroxy-3-(7-hydroxy-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-1-pyridin-4-ylmethyl-1H-quinolin-2-one, 1-(2-cyclopropylethyl)-6-fluoro-4-hydroxy-3-(7-(5-methyl-[1,3,4]oxadiazol-2-ylmethoxy)-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-1H-quinolin-2-one, 6-chloro-4-hydroxy-3-(7-hydroxy-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-1-(3-methylbutyl)-1H-quinolin-2-one, {3-[6-chloro-4-hydroxy-1-(3-methylbutyl)-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-7-yloxy}acetonitrile, 2-{3-[6-chloro-4-hydroxy-1-(3-methylbutyl)-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-7-yloxy}acetamide, 3-(7-amino-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-6-fluoro-4-hydroxy-1-(3-methylbutyl)-1H-quinolin-2-one, {3-[6-amino-4-hydroxy-1-(3-methylbutyl)-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-7-yloxy}acetonitrile, 3-[1-(2-cyclopropylethyl)-6-fluoro-4-hydroxy-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-7-yloxy}acetic acid, 2-{3-(7-carbamoylmethoxy-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-2-oxo-1,2-dihydroquinolin-6-ylamino}acetamide, 6-fluoro-4-hydroxy-3-(7-hydroxy-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-1-pyridin-4-ylmethyl-1H-quinolin-2-one, 1-(2-cyclopropylethyl)-6-fluoro-4-hydroxy-3-(7-(5-methyl-[1,3,4]oxadiazol-2-ylmethoxy)-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-1H-quinolin-2-one, 6-chloro-4-hydroxy-3-(7-hydroxy-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-1-(3-methylbutyl)-1H-quinolin-2-one, {3-[6-chloro-4-hydroxy-2-oxo-1,3-methylbutyl)-2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-7-yloxy}acetamide, and {3-[6-amino-4-hydroxy-1-(3-methylbutyl)-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-7-yloxy}acetonitrile, or a tautomer thereof, or a pharmaceutically acceptable salt or solvate thereof.

10. A pharmaceutically acceptable salt of the compound according to claim 9, or tautomer thereof, wherein said pharmaceutically acceptable salt is a sodium salt or a potassium salt.

11. A compound selected from the group consisting of:

3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(2-phenylethyl)-1H-quinolin-2-one, 1-(2-cyanobenzyl)-3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1H-quinolin-2-one, 3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-phenylpropyl)-1H-quinolin-2-one, N-[3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-2-oxo-1,2-dihydroquinolin-6-yl]4-methoxybenzamide, 3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-2-oxo-1,2-dihydroquinoline-6-carboxylic acid ethyl ester, 3-[1-(2-cyclopropylethyl)-4-hydroxy-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazine-7-carboxylic acid [2-(4-methoxyphenyl)ethyl]amide, 4-[3-(1,1-dioxo-1,2-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-2-oxoquinolin-1-yl]butyramide, 3-(1,1-dioxo-1,2-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-2-oxo-1,2-dihydroquinoline-7-carboxylic acid, 7-bromo-3-(1,1-dioxo-1,2-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-1H-quinolin-2-one, 3-(1,1-dioxo-7-phenyl-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-1H-quinolin-2-one, thiophene-2-carboxylic acid [3-(1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-2-oxo-1,2-dihydroquinolin-6-yl]amide, N-[3-(1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-2-oxo-1,2-dihydroquinolin-6-yl]-3-trifluorobenzamide, N-[3-(1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-2-oxo-1,2-dihydroquinolin-6-yl]-3-fluorobenzamide, N-[3-(1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-2-oxo-1,2-dihydroquinolin-6-yl]-nicotinamide, N-[3-(1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-2-oxo-1,2-dihydroquinolin-6-yl]acetamide, 4-hydroxy-3-(7-iodo-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-1-(3-methylbutyl)-1H-quinolin-2-one,

oxo-2H-quinolin-1-yl)-butyric acid ethyl ester, 4-[3-(1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-2-oxo-2H-quinolin-1-yl]-butyric acid, [3-(1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-6-(3-methyl-butoxy)-2-oxo-2H-quinolin-1-yl]acetic acid, 3-(1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-6-iodo-1-(3-methylbutyl)-1H-quinolin-2-one, 3-(1,1-dioxo-1,1-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-8-(3-methylbutyl)-1H-quinolin-2-one, 3-(1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-2-oxo-1,2-dihydro-quinoline-5-carboxylic acid dimethylamide, 3-(1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-2-oxo-1,2-dihydro-quinoline-5-carboxylic acid, 3-(1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-6-(4-methoxy-benzoyloxy)-1-(3-methylbutyl)-1H-quinolin-2-one, 1-(2-dimethylamino-ethyl)-3-(1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1H-quinolin-2-one, 4-hydroxy-3-(6-methoxy-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-1-(3-methylbutyl)-1H-quinolin-2-one, 6-(6-bromo-pyridin-2-yloxy)-3-(1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-1H-quinolin-2-one, 3-(1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(2-hydroxyethyl)-1H-quinolin-2-one, 3-(1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-6-(6-methoxy-pyridin-2-yloxy)-1-(3-methylbutyl)-1H-quinolin-2-one, 3-(1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-1-(5-fluoro-2-methyl-benzyl)-4-hydroxy-1H-quinolin-2-one, and 3-(1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(2-nitrobenzyl)-1H-quinolin-2-one, or a tautomer thereof, or a pharmaceutically acceptable salt or solvate thereof.

12. A pharmaceutically acceptable salt of the compound according to claim 11, or tautomer thereof, wherein said pharmaceutically acceptable salt is a sodium salt or a potassium salt.

13. A method of inhibiting an RNA-containing virus which comprises contacting said virus with an effective amount of the compound according to any one of claims 1 to 12.

14. A method of treating infection caused by an RNA-containing virus which comprises administering to a subject in need thereof an effective amount of the compound according to any one of claims 1 to 10.

15. A method according to claim 14 comprising treating an HCV infection.

16. A method according to claim 13 or claim 14 comprising inhibiting hepatitis C virus.

17. A method according to claim 15, wherein said HCV infection is acute hepatitis infection, chronic hepatitis infection, hepatocellular carcinoma or liver fibrosis.

18. A method according to claim 14 comprising treating an infection caused by Dengue, HIV or a picornavirus.

19. A method according to claim 14 comprising administering said compound in combination with one or more agents selected from the group consisting of an immunomodulatory agent and an antiviral agent.

20. A method according to claim 19, wherein the immunomodulatory agent is selected from the group consisting of alpha interferon, beta interferon, gamma interferon, a cytokine, a vitamin, a nutritional supplement, an antioxidant compound, a vaccine and a vaccine comprising an antigen and an adjuvant.

21. A method according to claim 14 comprising administering said compound in combination with an interferon.

22. A method according to claim 14 comprising administering said compound in combination with an HCV antisense agent.

23. A method according to claim 14 comprising administering said compound in combination with an immunoglobulin, a peptide-nucleic acid conjugate, an **oligonucleotide**, a ribozyme, a polynucleotide, an anti-inflammatory agent, a pro-inflammatory agent, an antibiotic or a hepatoprotectant.

24. A method for inhibiting replication of hepatitis C virus comprising inhibiting replication of both positive and negative strand HCV-RNA, said method comprising contacting a cell infected with said virus with an effective amount of the compound according to any one of claims 1 to 12.

25. A method of treating infection caused by hepatitis C virus comprising inhibiting replication of both positive and negative strand

thereof an effective amount of the compound according to any one of claims 1 to 10.

26. The method according to claim 24, wherein said compound substantially equally inhibits positive strand HCV-RNA replication and negative strand HCV-RNA replication.

27. The method according to claim 25, wherein said compound substantially equally inhibits positive strand HCV-RNA replication and negative strand HCV-RNA replication.

28. A method of preparing the compound of Formula I according to claim 1 comprising the steps of: a) treating a 2-aminobenzoic acid having the formula: ##STR15## with phosgene or a phosgene equivalent to provide a benzo[d][1,3]oxazine having the formula: ##STR16## or treating an indole-2,3-dione having the formula: ##STR17## with a peracid to provide the benzo[d][1,3]oxazine having the formula: ##STR18## b) converting the benzo[d][1,3]oxazine to an N-alkylated benzo[d][1,3]oxazine-2,4-dione having the formula: ##STR19## c) coupling the N-alkylated benzo[d][1,3]oxazine with a thiadiazine having the formula: ##STR20## where R is C₁-C₄ alkyl, to provide the compound of Formula I.

29. A method of preparing the compound of Formula I according to claim 1 comprising the steps of: a) treating a benzo[d][1,3]oxazine-2,4-dione having the formula: ##STR21## with a cyanoacetate to provide a 3-cyanoquinoline having the formula: ##STR22## b) treating the 3-cyanoquinoline with a 2-aminobenzenesulfonamide to provide the compound of Formula I, or converting the 3-cyanoquinoline to an amidine having the formula: ##STR23## and treating the amidine with a 2-chlorobenzenesulfonyl chloride to provide the compound of Formula I.

30. A method of preparing the compound of Formula I according to claim 1 comprising the steps of: a) treating an aniline with chlorosulfonylisocyanate then an acid to provide a thiadiazine compound having the formula: ##STR24## b) treating the thiadiazine compound with an aqueous acid to provide a 2-aminobenzenesulfonamide having the formula: ##STR25## c) treating the 2-aminobenzenesulfonamide with ethyl chloromalonate in the presence of a base to provide an amide having the formula: ##STR26## where R is C₁-C₄ alkyl, d) treating the amide with a dehydrating agent to provide a thiadiazines having the formula: ##STR27## e) treating the thiadiazine with a benzo[d][1,3]oxazine having the formula: ##STR28## to provide the compound of Formula I.

31. A method of preparing the compound of Formula I according to claim 1 comprising the steps of: a) converting a 7-methoxythiadiazine having the formula: ##STR29## where R is C₁-C₄ alkyl, to a 7-hydroxy compound having the formula: ##STR30## b) treating the 7-hydroxy compound with a benzo[d][1,3]oxazine having the formula: ##STR31## to provide a hydroxy-containing compound of Formula I having the formula: ##STR32## c) optionally treating the hydroxy-containing compound of Formula I with an agent to provide a compound of Formula I having the formula: ##STR33## where R⁹' is defined as R⁹, above, except that R⁹' is not H.

32. A method of preparing the compound of Formula I according to claim 1 comprising the steps of: a) treating a 2-aminobenzoic acid having the formula: ##STR34## with an aldehyde, having the formula W-CHO, in the presence of a reducing agent to provide an N-alkylated 2-aminobenzoic acid having the formula: ##STR35## b) treating the N-alkylated 2-aminobenzoic acid with phosgene or a phosgene equivalent to provide an N-alkylated benzo[d][1,3]oxazine-2,4-dione having the formula: ##STR36## c) coupling the N-alkylated benzo[d][1,3]oxazine with a thiadiazine having the formula: ##STR37## where R is C₁-C₄ alkyl, to provide the compound of Formula I.

33. A method of preparing the compound of Formula I according to claim 1 comprising the steps of: a) treating a 2-halobenzoic acid having the formula: ##STR38## where the halogen is bromine or chlorine, with an N-substituted amine, having the formula W--NH, in the presence of a copper catalyst to form an N-alkylated 2-aminobenzoic acid having the formula: ##STR39## b) converting the N-alkylated 2-aminobenzoic acid into the compound of Formula I.

34. The method I according to claim 33, further comprising the steps of: a) treating the N-alkylated 2-aminobenzoic acid with phosgene or a phosgene equivalent to provide an N-alkylated benzo[d][1,3]oxazine-2,4-dione having the formula: ##STR40## b) coupling the N-alkylated benzo[d][1,3]oxazine with a thiadiazine having the formula: ##STR41## where R is C₁-C₄ alkyl, to provide the compound of Formula I.

comprising the steps of: a) treating a 2-halobenzoic acid having the formula: ##STR42## where the halogen is bromine or chlorine, with an N-substituted amine, having the formula W--NH, in the presence of a catalyst to form an N-alkylated 2-aminobenzoic acid having the formula: ##STR43## b) treating the N-alkylated 2-aminobenzoic acid with phosgene or a phosgene equivalent to provide an N-alkylated benzo[d][1,3]oxazine-2,4-dione having the formula: ##STR44## c) coupling the N-alkylated benzo[a][1,3]oxazine with a thiadiazine having the formula: ##STR45## where R is C₁-C₄ alkyl, to provide the compound of Formula I. .

36. The method according to any one of claims 28-35 further comprising the step of treating the compound of Formula I with a base to provide the pharmaceutically acceptable salt of the compound of Formula I.

37. The method according to claim 36 wherein said base is sodium hydroxide or potassium hydroxide.

38. The method according to claim 36 wherein said pharmaceutically acceptable salt is a sodium salt or a potassium salt.

39. A compound having the formula: ##STR46## wherein R² is hydrogen halogen, --OR^b, --NHR^b, NO₂, where R^b is H or C₁-C₂ alkyl, where the C₁-C₂ alkyl is optionally unsubstituted or substituted by a substituent selected from the group consisting of cyano, --OH, --CO₂H, --CONH₂, --C(O)OC₁-C₂ alkyl, --CONH(C₁-C₂ alkyl), and unsubstituted monocyclic heteroaryl, or a pharmaceutically acceptable salt or solvate thereof.

40. A compound having the formula: ##STR47## wherein R² is hydrogen halogen, --OR^b, --NHR^b, NO₂, where R^b is H or C₁-C₂ alkyl, where the C₁-C₂ alkyl is optionally unsubstituted or substituted by a substituent selected from the group consisting of cyano, --OH, --CO₂H, --CONH₂, --C(O)OC₁-C₂ alkyl, --CONH(C₁-C₂ alkyl), and unsubstituted monocyclic heteroaryl, or a pharmaceutically acceptable salt or solvate thereof.

41. The compound according to claim 39 or claim 40, wherein R² is fluoro.

42. A compound selected from the group consisting of:
1-phenethyl-1H-benzo[d][1,3]oxazine-2,4-dione, 1-(2-cyanobenzyl)-1H-benzo[d][1,3]oxazine-2,4-dione, 1-(3-phenylpropyl)-1H-benzo[d][1,3]oxazine-2,4-dione, 1-cyclopropylmethyl-1H-benzo[d][1,3]oxazine-2,4-dione, 1-(3-methylbutyl)-6-nitrobenzo[d][1,3]oxazine-2,4-dione, 6-chloro-1-(3-methylbutyl)benzo[d][1,3]oxazine-2,4-dione, 6-bromo-1-(3-methylbutyl)benzo[d][1,3]oxazine-2,4-dione, 6-methoxy-3-methylbutylbenzo[d][1,3]oxazine-2,4-dione, 1-but-3-enyl-1H-benzo[d][1,3]oxazine-2,4-dione, 1-cyclohexylmethyl-1H-benzo[d][1,3]oxazine-2,4-dione, 1-(3-methylbut-2-enyl)-1H-benzo[d][1,3]oxazine-2,4-dione, 1-(3-methylbutyl)-6-methylbenzo[d][1,3]oxazine-2,4-dione, 1-(3-methylbutyl)-6-fluorobenzo[d][1,3]oxazine-2,4-dione, 1-(3-methylbutyl)-5-fluorobenzo[d][1,3]oxazine-2,4-dione, 1-(3-methylbutyl)-6,7-difluorobenzo[d][1,3]oxazine-2,4-dione, 1-(3-methylbutyl)-7-fluorobenzo[d][1,3]oxazine-2,4-dione, 1-(4,4,4-trifluorobutyl)-1H-benzo[d][1,3]oxazine-2,4-dione, 3-cyano-4-hydroxy-1-(3-methylbutyl)-1H-quinolin-2-one, 4-hydroxy-1-(3-methylbutyl)-2-oxo-1,2-dihydroquinoline-3-carboxamide, 1-pent-4-ynyl-1H-benzo[d][1,3]oxazine-2,4-dione, methyl (7-bromo-1,1-dioxo-1,2-dihydrobenzo[1,2,4]thiadiazin-3-yl)acetate, 8-bromo-2-isobutoxybenzo[d][1,3]oxazin-4-one, 1-cyclopropylmethyl-1H-benzo[d][1,3]oxazine-2,4-dione, 1-(3-methylbutyl)-5-methylbenzo[d][1,3]oxazine-2,4-dione, 1-(3-methylbutyl)-5-chlorobenzo[d][1,3]oxazine-2,4-dione, 5-bromo-2H-3,1-benzoxazine-1-(3-methylbutyl)-2,4-dione, 7-bromo-2H-3,1-benzoxazine-1-(3-methylbutyl)-2,4-dione, 4-(6-methoxybenzo[] [1,3]oxazine-2,4-dione-1-yl)butyronitrile, 1-butyne-3-yl-1H-benzo[d][1,3]oxazine-2,4-dione, 1-(3,3-dimethylbutyl)-1H-benzo[d][1,3]oxazine-2,4-dione, 1-2-cyclopropylethyl-1H-benzo[d][1,3]oxazine-2,4-dione, 3-cyano-4-hydroxy-1-(3-methylbutyl)-1H-quinolin-2-one, 1-(2-methylthio)ethyl-1H-benzo[d][1,3]oxazine-2,4-dione, 1-(2-methylthio)propyl-1H-benzo[d][1,3]oxazine-2,4-dione, 1-(3-furanylmethyl)methyl-1H-benzo[d][1,3]oxazine-2,4-dione, 1-[2-(2-thienyl)]ethyl-1H-benzo[d][1,3]oxazine-2,4-dione, 1-[2-(3-thienyl)]ethyl-1H-benzo[d][1,3]oxazine-2,4-dione, 1-(2-cyclopropylethyl)-6-fluorobenzo[d][1,3]oxazine-2,4-dione, 6-(tert-butyl-dimethylsilyloxy)-1-(2-cyclopropylethyl)-1H-benzo[d]oxazine-2,4-dione, 3-cyano-1-(2-cyclopropylethyl)-4-hydroxy-1H-quinolin-2-one, (7-iodo-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-acetic acid ethyl ester, 1-(3,3-dimethylbutyl)-6-

dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-6-nitro-1H-quinolin-2-one, 3-[4-hydroxy-1-(3-methylbutyl)-6-nitro-2-oxo-1,2-dihydroquinolin-3-yl]-1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazine-7-carboxylic acid amide, 1-(tetrahydrofuran-3-ylmethyl)-1H-benzo[d][1,3]oxazine-2,4-dione, 6-iodo-1-(3-methylbutyl)-1H-benzo[d][1,3]oxazine-2,4-dione, 1-(tetrahydro-furan-2-ylmethyl)-1H-benzo[d][1,3]oxazine-2,4-dione, 1-(3-methylpentyl)-1H-benzo[d][1,3]oxazine-2,4-dione, 4-hydroxy-3-(7-iodo-1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-1-(3-methylbutyl)-1H-quinolin-2-one, 5,6-dimethoxy-1-(3-methyl-butyl)-1H-benzo[d][1,3]oxazine-2,4-dione, 4-methyl-1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-one, N-methyl-N-(2-sulfamoylphenyl)malonamic acid ethyl ester, (4-methyl-1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)acetic acid ethyl ester, 3-(1,1-dioxo-7-methyl-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-acetic acid ethyl ester, 1-(4-nitrobenzyl)-1H-benzo[d][1,3]oxazine-2,4-dione, 1-(6-aminopyridin-3-ylmethyl)-1H-benzo[d][1,3]oxazine-2,4-dione, 1-(4-bromobenzyl)-1H-benzo[d][1,3]oxazine-2,4-dione, 1-(3-bromobenzyl)-1H-benzo[d][1,3]oxazine-2,4-dione, 7-methoxy-1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-one, N-(4-methoxy-2-sulfamoylphenyl)malonic acid ethyl ester, (7-methoxy-1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-acetic acid ethyl ester, 1-(2-cyclopropylethyl)-3-(1,1-Dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-7-iodo-3-yl)-6-fluoro-4-hydroxy-1H-quinolin-2-one, (7-hydroxy-1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)acetic acid ethyl ether, 1-([2-methylcyclopropyl)methyl]-1H-benzo[d][1,3]oxazine-2,4-dione, 1-([2-methylcyclopropyl)methyl]-1H-benzo[d][1,3]oxazine-2,4-dione, 4,6-dihydroxy-3-(7-methoxy-1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-1-(3-methyl-butyl)-1H-quinolin-2-one, 1-(2-cyclopropyl-ethyl)-4,6-dihydroxy-3-(7-hydroxy-1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]thiadiazin-3-yl)-1H-quinolin-2-one, 6-amino-1-(3-methylbutyl)-1H-benzo[d][1,3]oxazine-2,4-dione, 6-amino-4-hydroxy-3-(7-methoxy-1,1-dioxo-1,2-dihydrobenzo[1,2,4]thiadiazin-3-yl)-1-(3-methylbutyl)-1H-quinolin-2-one, 6-amino-4-hydroxy-3-(7-hydroxy-1,1-dioxo-1,2-dihydrobenzo[1,2,4]thiadiazin-3-yl)-1-(3-methylbutyl)-1H-quinolin-2-one, and 1-(3-nitrobenzyl)-1H-benzo[d][1,3]oxazine-2,4-dione, 1-[(2-methylpyridin-4yl)methyl]-1H-benzo[d][1,3]oxazine-2,4-dione, or a tautomer thereof, or a pharmaceutically acceptable salt or solvate thereof.

L5 ANSWER 9 OF 112 USPTAFULL on STN

2004:171478 Novel composition.

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US 2004131638 A1 20040708

APPLICATION: US 2003-475784 A1 20031023 (10)

WO 2002-EP4966 20020425

PRIORITY: GB 2001-10431 20010427

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to a vaccine composition comprising at least one human immunodeficiency virus (HIV) antigen and either one or both of: i) at least one herpes simplex virus (HSV) antigen and ii) at least one human papillomavirus (HPV) antigen.

CLM What is claimed is:

1. A vaccine composition comprising: (a) at least one human immunodeficiency virus (HIV) antigen; and either one or both of (b) at least one herpes simplex virus (HSV) antigen and (c) at least one human papillomavirus (HPV) antigen.

2. A vaccine composition as claimed in claim 1 wherein the HIV antigen is selected from the group consisting of; gp160, gp120, nef, tat, a nef-tat or tat-nef fusion protein, gag, pol or immunologically active derivatives thereof.

3. A vaccine composition as claimed in claim 2 wherein the vaccine comprises HIV antigens gp120 and a nef-tat fusion protein.

4. A vaccine composition according to claim 2 or 3 wherein the Tat, Nef or Nef-tat act in synergy with gp120.

5. A vaccine composition according to any preceding claim wherein the HPV antigen is selected from the group consisting of L1, L2, E6 and E7 or combinations thereof, optionally in the form of a fusion protein or a truncate..

6. A vaccine composition as claimed in claim 5 wherein the HPV antigen is a virus like particle comprising the L1 protein or a C terminal truncation thereof.

HSV antigen is HSV-2 gD or a truncate thereof

8. A vaccine composition as claimed in any one of the preceding claims which further comprises an adjuvant.

9. A vaccine composition according to claim 8 wherein the adjuvant is a preferential stimulator of TH1-cell response.

10. A vaccine composition according to claim 9 wherein the preferential stimulator of TH1-cell response is selected from the group of adjuvants comprising: 3D-MPL, 3D-MPL wherein the size of the particles of 3D-MPL is preferably about or less than 100 nm, QS21, a mixture of QS21 and cholesterol and a CpG **oligonucleotide**, or combinations thereof.

11. A composition according to claim 9 or 10 which additionally comprises an oil in water emulsion.

12. A vaccine composition according to claim 11 comprising HIV gp120 and a fusion protein of HIV Nef with HIV Tat in combination with QS21, 3D-MPL and an oil-in-water emulsion.

13. A vaccine composition according to any preceding claim wherein at least one antigen is in the form of DNA or a live vector.

14. A vaccination kit comprising: (a) at least one human immunodeficiency virus (HIV) antigen; and either one or both of (b) at least one herpes simplex virus (HSV) antigen; and (c) at least one human papillomavirus (HPV) antigen.

15. A method of medical treatment comprising delivering to an individual in need of such treatment an effective amount of a vaccine against HIV and HSV and/or HPV.

16. A method according to claim 15, comprising the delivery of a vaccine against HIV and HSV.

17. A method according to claim 15, comprising the delivery of a vaccine against HIV and HPV.

18. A method according to any of claims 15 to 17 comprising delivery of a single vaccine containing a mixture of antigens from HIV and HSV and/or HPV.

19. A method according to any of claims 15 to 17 wherein vaccines against HIV and HSV and/or HPV are co-administered at separate administration sites.

20. Use of an BPV antigen in the preparation of a medicament for the prevention or treatment of HIV or HSV infection or disease.

21. Use of an HSV antigen in the preparation of a medicament for the prevention or treatment of HIV or HPV infection or disease.

22. Use according to any of claims 20 or 21 wherein the use is for prevention or treatment of HIV infection or disease.

23. A method for the preparation of a vaccine according to any of claims 1-13 comprising combining at least one human immunodeficiency virus (HIV) antigen with either one or both of: i) at least one herpes simplex virus (HSV) antigen; and ii) at least one human papillomavirus (HPV) antigen.

24. A method of decreasing HIV viral transmission, the method comprising treatment with a vaccine according to any of claims 1-13.

L5 ANSWER 10 OF 112 USPTAFULL on STN

2004:164901 Serotype and dengue group specific fluorigenic probe based PCR (TaqMan) assays against the respective C and NS5 genomic and 3' non-coding regions of **dengue virus**.

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US 2004126387 A1 20040701

APPLICATION: US 2000-726345 A1 20001201 (9)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Five fluorogenic probe hydrolysis reverse transcriptase-**polymerase chain reaction** (RT-PCR) (TaqMan.TM.) assays are described for serotype-specific detection of dengue 1-4 and group-specific detection of dengue viruses. Type- and group-specific oligonucleotide primers and fluorogenic probes were designed against conserved regions of the dengue genome. The invention provides TaqMan **PCR** assays, which are rapid,

epidemiological study of dengue infections.

What is claimed is:

1. A forward **oligonucleotide primer** for dengue-1 virus consisting of the sequence 5'-GAC ACC ACA CCC TTT GGA CAA-3'
2. A reverse **oligonucleotide primer** for dengue-1 virus consisting of the sequence 5'-CAC CTG GCT GTC ACC TCC AT-3'
3. An **oligonucleotide probe** for dengue-1 virus-consisting of the sequence 5'-AGA GGG TGT TTA AAG AGA AAG TTG ACA CGC G-3'
4. A forward **oligonucleotide primer** for dengue-2 virus consisting of the sequence 5'-CCG CGT GTC GAC TGT ACA A-3'
5. A reverse **oligonucleotide primer** for dengue-2 virus consisting of the sequence 5'-CAG GGC CAT GAA CAG TTT TAA-3'
6. An **oligonucleotide probe** for dengue-2 virus consisting of the sequence 5'-TTG GAA TGC TGC AGG GGA CGA GGA-3'
7. A forward **oligonucleotide primer** for dengue-3 virus consisting of the sequence 5'-GGG AAA ACC GTC TAT CAA TA-3'
8. A reverse **oligonucleotide primer** for dengue-3 virus consisting of the sequence 5'-CGC CAT AAC CAA TTT CAT TGG-3'
9. An **oligonucleotide probe** for dengue-3 virus consisting of the sequence 5'-CAC AGT TGG CGA AGA GAT TCT CAA CAG GA-3'
10. A forward **oligonucleotide primer** for dengue-4 virus consisting of the sequence 5'-TGA AGA GAT TCT CAA CCG GAC-3'
11. A reverse **oligonucleotide primer** for dengue-4 virus consisting of the sequence 5'-AAT CCC TGC TGT TGG TGG G-3'
12. An **oligonucleotide probe** for dengue-4 virus consisting of the sequence 5'-TCA TCA CGT TTT TGC GAG TCC TTT CCA-3'
13. A group specific forward **oligonucleotide primer** for **dengue virus** consisting of the sequence 5'-AAG GAC TAG AGG TTA KAG GAG ACC C-3'
14. A group specific reverse **oligonucleotide primer** for **dengue virus** consisting of the sequence 5'-GGC GYT CTG TGC CTG GAW TGA TG-3'
15. A group specific **oligonucleotide probe** for **dengue virus** serotypes 1 and 3 consisting of the sequence 5'-FAM-AAC AGC ATA TTG ACG CTG GGA GAG ACC-TAMRA-3'
16. A group specific **oligonucleotide probe** for **dengue virus** serotypes 2 and 4 consisting of the sequence 5'-MAX-AAC AGC ATA TTG ACG CTG GGA AAG ACC-TAMRA-3'
17. A method of detecting the presence of **Dengue virus** by **polymerase chain reaction**, said method comprising: a) providing the RNA of said **Dengue virus** or a test sample of RNA suspected of being **Dengue virus** RNA, RT enzymes, dATPs, dGTPs, dCTPs, dTTPs and buffer containing divalent cations such as magnesium cation in sufficient quantities so reverse transcription of a cDNA copy occurs, b) providing group specific or serotype-specific **primers** and probes of **Dengue** in sufficient quantities so amplification of a target sequence of DNA occurs, c) detecting the presence of the amplification products of the target sequence of DNA as an indication of the presence of **Dengue virus**.
18. A method of detecting the presence of Dengue-1 virus by **polymerase chain reaction**, said method comprising: a) providing the RNA of said Dengue-1 virus or a test sample of RNA suspected of being Dengue-1 virus RNA, RT enzymes, dATPs, dGTPs, dCTPs, dTTPs and buffer containing divalent cations in sufficient quantities so reverse transcription of a cDNA copy occurs, b) providing serotype-specific **primers** and a probe for Dengue-1 virus in sufficient quantities so amplification of a target sequence of DNA occurs, c) detecting the presence of the amplification products of the target sequence of DNA as an indication of the presence of Dengue-1 virus.
19. A method of detecting the presence of Dengue-2 virus by **polymerase chain reaction**, said method comprising: a) providing the RNA of said Dengue-2 virus or a test sample of RNA suspected of being Dengue-1 virus RNA, RT enzymes, dATPs, dGTPs, dCTPs, dTTPs and buffer containing divalent cations in sufficient quantities so reverse transcription of a cDNA copy occurs, b) providing serotype-specific **primers** and a probe for Dengue-2 virus in sufficient quantities so amplification of a target

products of the target sequence of DNA as an indication of the presence of Dengue-2 virus.

20. A method of detecting the presence of Dengue-3 virus by **polymerase chain reaction**, said method comprising: a) providing the RNA of said Dengue-3 virus or a test sample of RNA suspected of being Dengue-1 virus RNA, RT enzymes, dATPs, dGTPs, dCTPs, dTTPs and buffer containing divalent cations in sufficient quantities so reverse transcription of a cDNA copy occurs, b) providing serotype-specific **primers** and a probe for Dengue-3 virus in sufficient quantities so amplification of a target sequence of DNA occurs, c) detecting the presence of the amplification products of the target sequence of DNA as an indication of the presence of Dengue-3 virus.

21. A method of detecting the presence of Dengue-4 virus by **polymerase chain reaction**, said method comprising: a) providing the RNA of said Dengue-4 virus or a test sample of RNA suspected of being Dengue-1 virus RNA, RT enzymes, dATPs, dGTPs, dCTPs, dTTPs and buffer containing divalent cations in sufficient quantities so reverse transcription of a cDNA copy occurs, b) providing serotype-specific **primers** and a probe for Dengue-4 virus in sufficient quantities so amplification of a target sequence of DNA occurs, c) detecting the presence of the amplification products of the target sequence of DNA as an indication of the presence of Dengue-4 virus.

L5 ANSWER 11 OF 112 USPTAFULL on STN

2004:160052 Secondary structure defining database and methods for determining identity and geographic origin of an unknown bioagent in forensic studies thereby.

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Griffey, Richard, Vista, CA, UNITED STATES

Sampath, Rangarajan, San Diego, CA, UNITED STATES

Hofstadler, Steven, Oceanside, CA, UNITED STATES

McNeil, John, La Jolla, CA, UNITED STATES

Crooke, Stanley T., Carlsbad, CA, UNITED STATES

US 2004122857 A1 20040624

APPLICATION: US 2002-326643 A1 20021218 (10)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates generally to the field of investigational bioinformatics and more particularly to secondary structure defining databases. The present invention further relates to methods for interrogating a database as a source of molecular masses of known bioagents for comparing against the molecular mass of an unknown or selected bioagent to determine either the identity of the selected bioagent, and/or to determine the origin of the selected bioagent. The identification of the bioagent is important for determining a proper course of treatment and/or eradication of the bioagent in such cases as biological warfare. Furthermore, the determination of the geographic origin of a selected bioagent will facilitate the identification of potential criminal identity.

CLM What is claimed is:

1. A database having cell-data positional significance comprising at least a first table of a plurality of data-containing cells, said first table organized into at least a first row and a second row, each row having columns and data-containing cells; and wherein said data-containing cells have an alignment with at least one other row for differentiating aligned from non-aligned data-containing cells, and wherein said differentiation in alignment of said data-containing cells designates a geographic feature of a polymer present in a sample.

2. The database according to claim 1 wherein said alignment is a vertical alignment according to base pair homology along a linear segment within each polymer.

3. The database according to claim 1 wherein said vertical alignment further aligns cell-data according to inter-species conserved regions.

4. The database according to claim 1 wherein the geographic feature is an origin of a bioagent.

5. The database according to claim 1 wherein the polymer is an RNA.

6. A service providing information related to the geography of a bioagent in a sample comprising: providing a dimensional master database for storing a molecular mass, an identity and a geographic detail corresponding to a plurality of known bioagents and, said master database storing the molecular mass, the identity and the geographic detail for a plurality of known bioagents; interrogating the master database with an identification request of an unknown bioagent in a sample to generate a response; and delivering said response from the

7. The service according to claim 6 wherein the molecular mass is of a selected portion of the known bioagent, the identity comprises at least a name for the known bioagent, and the geographic detail comprises at least a geographic origin, geographic distribution, or treatment.

8. The service according to claim 6 wherein the request comprises a symptomatology and the identification comprises a recommended pair of **primers** for hybridizing to sequences of nucleic acid flanking a variable nucleic acid sequence of the unknown bioagent, and said pair of **primers** are hybridized to the sequences of nucleic acid flanking a variable nucleic acid sequence of the unknown bioagent.

9. The service according to claim 8 wherein the nucleic acid sequence of the unknown bioagent between said pair of **primers** defines the selected portion of both the known bioagents and the unknown bioagent.

10. The service according to claim 9 wherein the response is delivered through a network.

11. The service according to claim 9 wherein the request comprises a molecular mass of the unknown bioagent for the selected portion and where the response generated thereto comprises a set of molecular masses for analogous selected portions of known bioagents, and said set comprising at least one molecular mass from the master database.

12. The service according to claim 10 wherein the network is a local area network.

13. The service according to claim 10 wherein the network is a wide area network.

14. The service according to claim 11 wherein the network is the internet.

L5 ANSWER 12 OF 112 USPTAFULL on STN

2004:159793 Secondary structure defining database and methods for determining identity and geographic origin of an unknown bioagent in food products and cosmetics thereby.

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Griffey, Richard, Vista, CA, UNITED STATES

Sampath, Rangarajan, San Diego, CA, UNITED STATES

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US 2004122598 A1 20040624

APPLICATION: US 2002-326046 A1 20021218 (10)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates generally to the field of investigational bioinformatics and more particularly to secondary structure defining databases. The present invention further relates to methods for interrogating a database as a source of molecular masses of known bioagents for comparing against the molecular mass of an unknown or selected bioagent to determine either the identity of the selected bioagent, and/or to determine the origin of the selected bioagent. The identification of the bioagent is important for determining a proper course of treatment and/or eradication of the bioagent in such cases as biological warfare. Furthermore, the determination of the geographic origin of a selected bioagent will facilitate the identification of potential criminal identity.

CLM What is claimed is:

1. A method of identifying an unknown bioagent in a food product or cosmetic sample using a database of molecular masses of known bioagents comprising: contacting nucleic acid from said bioagent in said food product or cosmetic sample with at least one pair of **oligonucleotide primers** that hybridize to sequences of said nucleic acid, wherein said sequences flank a variable nucleic acid sequence of said bioagent in said food product or cosmetic; producing an amplification product of said variable nucleic acid sequence; determining a first molecular mass of said amplification product; and comparing said first molecular mass to the molecular masses of known bioagents, thereby identifying the unknown bioagent in said food product or cosmetic sample.

2. The method of claim 1 wherein said sequences to which said at least one pair of **oligonucleotide primers** hybridize are highly conserved.

3. The method of claim 1 wherein said sequences to which said at least one pair of **oligonucleotide primers** hybridize are highly conserved across at least two species.

nucleic acid from said sample prior to contacting said nucleic acid with said at least one pair of **oligonucleotide primers**, wherein the comparing step further comprises comparing a base-pair count resulting from a translation of the corresponding molecular mass, and wherein a master database of molecular masses of known bioagents further includes a translation of said molecular masses of known bioagents to corresponding base-pair counts of each known bioagent resulting from a specific **primer** pair set and comparing the base-pair count of said unknown bioagent against the obtained base-pair count of known bioagents for the selected **primer** pair set for determining the identity of said unknown bioagent in said sample.

5. The method of claim 4 further comprising the step of reconciling the database of molecular masses of known bioagents with the master database of molecular masses of known bioagents.

6. The method of claim 1 wherein said bioagent is a bacterium, parasite, fungi, virus, cell or spore.

7. The method of claim 1 wherein said food product is a food susceptible to bacterial contamination.

8. The method of claim 7 wherein said food susceptible to bacterial contamination is a meat product, agricultural product, or egg.

9. The method of claim 1 wherein said bioagent is a pathogenic organism.

10. The method of claim 9 wherein said pathogenic organism is E. coli or a Salmonella species.

11. The method of claim 1 wherein said amplification product is ionized by electrospray ionization, matrix assisted laser desorption or fast atom bombardment prior to molecular mass determination.

12. The method of claim 1 wherein said molecular mass is determined by mass spectrometry.

13. The method of claim 5 wherein said master database of molecular masses of known bioagents and the database of molecular masses of known bioagents are reconciled over a network.

14. The method of claim 4 wherein the identity is determined by statistically correlating the molecular mass of the unknown bioagent with at least one molecular mass of said master database.

15. A database having cell-data positional significance comprising at least a first table of a plurality of data-containing cells, said first table organized into at least a first row and a second row, each row having columns and data-containing cells; and wherein said data-containing cells have an alignment with at least one other row for differentiating aligned from non-aligned data-containing cells, and wherein said differentiation in alignment of said data-containing cells designates a structural feature of a polymer present in a food product or cosmetic sample.

16. The database according to claim 15 wherein said alignment is a vertical alignment according to base pair homology along a linear segment within each polymer.

17. The database according to claim 15 wherein said vertical alignment further aligns cell-data according to inter-species conserved regions.

18. The database according to claim 15 wherein the structural feature is a bulge or a loop.

19. The database according to claim 15 wherein the polymer is an RNA.

20. The method of claim 15 wherein said food product is a food susceptible to bacterial contamination.

21. The method of claim 20 wherein said food susceptible to bacterial contamination is a meat product, agricultural product, or egg.

22. The method of claim 15 wherein said bioagent is a pathogenic organism.

23. The method of claim 22 wherein said pathogenic organism is E. coli or a Salmonella species.

24. A service providing information related to a bioagent in a food product or cosmetic sample comprising: providing a dimensional master database for storing a molecular mass, an identity and a detail

database storing the molecular mass, the identity and the detail for a plurality of known bioagents; interrogating the master database with an identification request of an unknown bioagent in a food product or cosmetic sample to generate a response; and delivering said response from the master database to a requester.

25. The service according to claim 24 wherein the molecular mass is of a selected portion of the known bioagent, the identity comprises at least a geographic origin and a name for the known bioagent, and the detail comprises at least a treatment.

26. The service according to claim 24 wherein the request comprises a symptomatology and the identification comprises a recommended pair of **primers** for hybridizing to sequences of nucleic acid flanking a variable nucleic acid sequence of the unknown bioagent, and said pair of **primers** are hybridized to the sequences of nucleic acid flanking a variable nucleic acid sequence of the unknown bioagent.

27. The service according to claim 26 wherein the nucleic acid sequence of the unknown bioagent between said pair of **primers** defines the selected portion of both the known bioagents and the unknown bioagent.

28. The service according to claim 27 wherein the response is delivered through a network.

29. The service according to claim 27 wherein the request comprises a molecular mass of the unknown bioagent for the selected portion and where the response generated thereto comprises a set of molecular masses for analogous selected portions of known bioagents, and said set comprising at least one molecular mass from the master database.

30. The service according to claim 28 wherein the network is a local area network.

31. The service according to claim 28 wherein the network is a wide area network.

32. The service according to claim 29 wherein the network is the internet.

33. A method of determining a geographical origin of a selected bioagent in a food product or cosmetic sample using a database of molecular masses of known bioagents comprising: contacting nucleic acid from said selected bioagent in said food product or cosmetic sample with at least one pair of **oligonucleotide primers** which hybridize to sequences of said nucleic acid, wherein said sequences flank a variable nucleic acid sequence of the bioagent in said food product or cosmetic sample; producing an amplification product of said variable nucleic acid sequence; determining a first molecular mass of said amplification product; and comparing said first molecular mass to the molecular masses of known bioagents for determining a geographic origin of said selected bioagent, said comparison determining an identity and a geographic origin of said selected bioagent in said food product or cosmetic sample.

34. The method of claim 33 wherein said sequences to which said at least one pair of **oligonucleotide primers** hybridize are highly conserved.

35. The method of claim 33 wherein said sequences to which said at least one pair of **oligonucleotide primers** hybridize are highly conserved across species.

36. The method of claim 33 further comprising the step of isolating a nucleic acid from said selected bioagent prior to contacting said nucleic acid with said at least one pair of **oligonucleotide primers**, wherein the comparing step further comprises interrogating a master database of molecular masses of known bioagents for obtaining molecular masses of known bioagents and comparing the molecular mass of said selected bioagent against the obtained molecular masses of known bioagents thereby determining an origin of said selected bioagent.

37. The method of claim 36 further comprising the step of reconciling the database of molecular masses of known bioagents with the master database of molecular masses of known bioagents.

38. The method of claim 33 wherein said bioagent is a bacterium, parasite, fungi, virus, cell or spore.

39. The method of claim 33 wherein said food product is a food susceptible to bacterial contamination.

40. The method of claim 39 wherein said food susceptible to bacterial

41. The method of claim 39 wherein said bioagent is a pathogenic organism.
42. The method of claim 41 wherein said pathogenic organism is *E. coli* or a *Salmonella* species.
43. The method of claim 33 wherein said amplification product is ionized by electrospray ionization, matrix-assisted laser desorption or fast atom bombardment prior to molecular mass determination.
44. The method of claim 33 wherein said molecular mass is determined by mass spectrometry.
45. The method of claim 36 wherein said master database of molecular masses of known bioagents and the database of molecular masses of known bioagents are reconciled over a network.
46. The method of claim 36 wherein the origin comprises a statistical group of matching molecular masses and the geographic origin corresponding thereto.

L5 ANSWER 13 OF 112 USPTFULL on STN

2004:158541 Secondary structure defining database and methods for determining identity and geographic origin of an unknown bioagent associated with host versus graft and graft versus host rejections thereby.

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 Griffey, Richard, Vista, CA, UNITED STATES
 Sampath, Rangarajan, San Diego, CA, UNITED STATES
 Hofstadler, Steven, Oceanside, CA, UNITED STATES
 McNeil, John, La Jolla, CA, UNITED STATES
 Crooke, Stanley T., Carlsbad, CA, UNITED STATES
 US 2004121340 A1 20040624

APPLICATION: US 2002-326641 A1 20021218 (10)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates generally to the field of investigational bioinformatics and more particularly to secondary structure defining databases. The present invention further relates to methods for interrogating a database as a source of molecular masses of known bioagents for comparing against the molecular mass of an unknown or selected bioagent to determine either the identity of the selected bioagent, and/or to determine the origin of the selected bioagent. The identification of the bioagent is important for determining a proper course of treatment and/or eradication of the bioagent in such cases as biological warfare. Furthermore, the determination of the geographic origin of a selected bioagent will facilitate the identification of potential criminal identity.

CLM What is claimed is:

1. A method of identifying a major histocompatibility complex antigen in an organ recipient or donor organ using a database of molecular masses of known bioagents comprising: contacting nucleic acid encoding said major histocompatibility complex antigen in an organ recipient or donor organ with at least one pair of **oligonucleotide primers** that hybridize to sequences of said nucleic acid, wherein said sequences flank a variable nucleic acid sequence of said major histocompatibility complex antigen in an organ recipient or donor organ; producing an amplification product of said variable nucleic acid sequence; determining a first molecular mass of said amplification product; and comparing said first molecular mass to the molecular masses of known bioagents, thereby identifying the unknown major histocompatibility complex antigen in an organ recipient or donor organ.

2. The method of claim 1 wherein said sequences to which said at least one pair of **oligonucleotide primers** hybridize are highly conserved.

3. The method of claim 1 wherein said sequences to which said at least one pair of **oligonucleotide primers** hybridize are highly conserved across at least two species.

4. The method of claim 1 further comprising the step of isolating a nucleic acid from said organ recipient or donor organ prior to contacting said nucleic acid with said at least one pair of **oligonucleotide primers**, wherein the comparing step further comprises comparing a base-pair count resulting from a translation of the corresponding molecular mass, and wherein a master database of molecular masses of known major histocompatibility complex antigens further includes a translation of said molecular masses of known major histocompatibility complex antigens to corresponding base-pair counts of each known major histocompatibility complex antigen resulting from a specific **primer** pair set and comparing the base-pair count of said

base-pair count of known major histocompatibility complex antigens for the selected **primer** pair set for determining the identity of said unknown major histocompatibility complex antigen in said organ recipient or donor organ.

5. The method of claim 4 further comprising the step of reconciling the database of molecular masses of known major histocompatibility complex antigens with the master database of molecular masses of known major histocompatibility complex antigens.....

6. The method of claim 1 wherein said major histocompatibility complex antigen induces a graft versus host or host versus graft response.

7. The method of claim 1 wherein said nucleic acid is RNA.

8. The method of claim 1 wherein said nucleic acid encodes an HLA antigen.

9. The method of claim 1 wherein said amplification product is ionized by electrospray ionization, matrix assisted laser desorption or fast atom bombardment prior to molecular mass determination.

10. The method of claim 1 wherein said molecular mass is determined by mass spectrometry.

11. The method of claim 5 wherein said master database of molecular masses of known major histocompatibility complex antigens and the database of molecular masses of known major histocompatibility complex antigens are reconciled over a network.

12. The method of claim 4 wherein the identity is determined by statistically correlating the molecular mass of the unknown major histocompatibility complex antigen with at least one molecular mass of said master database.

13. A database having cell-data positional significance comprising at least a first table of a plurality of data-containing cells, said first table organized into at least a first row and a second row, each row having columns and data-containing cells; and wherein said data-containing cells have an alignment with at least one other row for differentiating aligned from non-aligned data-containing cells, and wherein said differentiation in alignment of said data-containing cells designates a structural feature of a polymer encoding a major histocompatibility complex antigen present in an organ recipient or donor organ.

14. The database according to claim 13 wherein said alignment is a vertical alignment according to base pair homology along a linear segment within each polymer.

15. The database according to claim 13 wherein said vertical alignment further aligns cell-data according to inter-species conserved regions.

16. The database according to claim 13 wherein the structural feature is a bulge or a loop.

17. The method of claim 13 wherein said major histocompatibility complex antigen induces a graft versus host or host versus graft response.

18. The method of claim 13 wherein said polymer encodes an HLA antigen.

19. The method of claim 13 wherein said polymer is RNA.

20. A service providing information related to a major histocompatibility complex antigen in an organ recipient or donor organ comprising: providing a dimensional master database for storing a molecular mass, an identity and a detail corresponding to a plurality of known major histocompatibility complex antigens and, said master database storing the molecular mass, the identity and the detail for a plurality of known major histocompatibility complex antigens; interrogating the master database with an identification request of an unknown major histocompatibility complex antigen in a sample to generate a response; and delivering said response from the master database to a requester.

21. The service according to claim 20 wherein the molecular mass is of a selected portion of the known major histocompatibility complex antigen, the identity comprises at least a geographic origin and a name for the known major histocompatibility complex antigen, and the detail comprises at least a treatment.

22. The service according to claim 20 wherein the request comprises a

primers for hybridizing to sequences of nucleic acid flanking a variable nucleic acid sequence of the unknown major histocompatibility complex antigen, and said pair of **primers** are hybridized to the sequences of nucleic acid flanking a variable nucleic acid sequence of the unknown major histocompatibility complex antigen.

23. The service according to claim 22 wherein the nucleic acid sequence of the unknown major histocompatibility complex antigen between said pair of **primers** defines the selected portion of both the known major histocompatibility complex antigens and the unknown major histocompatibility complex antigen.

24. The service according to claim 23 wherein the response is delivered through a network.

25. The service according to claim 23 wherein the request comprises a molecular mass of the unknown major histocompatibility complex antigen for the selected portion and where the response generated thereto comprises a set of molecular masses for analogous selected portions of known major histocompatibility complex antigens, and said set comprising at least one molecular mass from the master database.

26. The service according to claim 24 wherein the network is a local area network.

27. The service according to claim 24 wherein the network is a wide area network.

28. The service according to claim 25 wherein the network is the internet.

29. A method of determining a geographical origin of a selected major histocompatibility complex antigen in organ recipient or donor organ using a database of molecular masses of known major histocompatibility complex antigens comprising: contacting nucleic acid from said selected major histocompatibility complex antigen in said organ recipient or donor organ with at least one pair of **oligonucleotide primers** which hybridize to sequences of said nucleic acid, wherein said sequences flank a variable nucleic acid sequence of the major histocompatibility complex antigen in said organ recipient or donor organ; producing an amplification product of said variable nucleic acid sequence; determining a first molecular mass of said amplification product; and comparing said first molecular mass to the molecular masses of known major histocompatibility complex antigens for determining a geographic origin of said selected major histocompatibility complex antigen, said comparison determining an identity and a geographic origin of said selected major histocompatibility complex antigen in said organ recipient or organ donor.

30. The method of claim 29 wherein said sequences to which said at least one pair of **oligonucleotide primers** hybridize are highly conserved.

31. The method of claim 29 wherein said sequences to which said at least one pair of **oligonucleotide primers** hybridize are highly conserved across species.

32. The method of claim 29 further comprising the step of isolating a nucleic acid from said organ recipient or donor organ prior to contacting said nucleic acid with said at least one pair of **oligonucleotide primers**, wherein the comparing step further comprises interrogating a master database of molecular masses of known major histocompatibility complex antigens for obtaining molecular masses of known major histocompatibility complex antigens and comparing the molecular mass of said selected major histocompatibility complex antigen against the obtained molecular masses of known major histocompatibility complex antigen thereby determining an origin of said selected major histocompatibility complex antigen.

33. The method of claim 32 further comprising the step of reconciling the database of molecular masses of known major histocompatibility complex antigen with the master database of molecular masses of known major histocompatibility complex antigens.

34. The method of claim 29 wherein said major histocompatibility complex antigen induces a graft versus host or host versus graft response.

35. The method of claim 29 wherein said nucleic acid is RNA.

36. The method of claim 29 wherein said nucleic acid encodes an HLA antigen.

37. The method of claim 29 wherein said amplification product is ionized

atom bombardment prior to molecular mass determination.

38. The method of claim 29 wherein said molecular mass is determined by mass spectrometry.

39. The method of claim 32 wherein said master database of molecular masses of known major histocompatibility complex antigens and the database of molecular masses of known major histocompatibility complex antigens are reconciled over a network.

40. The method of claim 32 wherein the origin comprises a statistical group of matching molecular masses and the geographic origin corresponding thereto.

L5 ANSWER 14 OF 112 USPATFULL on STN

2004:158536 Methods for rapid detection and identification of bioagents associated with host versus graft and graft versus host rejections.
Ecker, David J., Encinitas, CA, UNITED STATES
Griffey, Richard H., Vista, CA, UNITED STATES
Sampath, Rangarajan, San Diego, CA, UNITED STATES
Hofstadler, Steven, Oceanside, CA, UNITED STATES
McNeil, John, La Jolla, CA, UNITED STATES
US 2004121335 A1 20040624

APPLICATION: US 2002-325527 A1 20021218 (10)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Method for detecting and identifying unknown bioagents, including bacteria, viruses and the like, by a combination of nucleic acid amplification and molecular weight determination using primers which hybridize to conserved sequence regions of nucleic acids derived from a bioagent and which bracket variable sequence regions that uniquely identify the bioagent. The result is a "base composition signature" (BCS) which is then matched against a database of base composition signatures, by which the bioagent is identified.

CLM What is claimed is:

1. A method of identifying a major histocompatibility complex antigen in an organ recipient or donor organ comprising: a) contacting nucleic acid encoding said major histocompatibility complex antigen in said organ recipient or donor organ with at least one pair of **oligonucleotide primers** which hybridize to sequences of said nucleic acid, wherein said sequences flank a variable nucleic acid sequence of the major histocompatibility complex antigen; b) amplifying said variable nucleic acid sequence to produce an amplification product; c) determining the molecular mass of said amplification product; and d) comparing said molecular mass to one or more molecular masses of amplification products obtained by performing steps a)-c) on a plurality of known organisms, wherein a match identifies said major histocompatibility complex antigen in said organ recipient or donor organ.

2. The method of claim 1 wherein said sequences to which said at least one pair of **oligonucleotide primers** hybridize are highly conserved.

3. The method of claim 1 wherein said amplifying step comprises **polymerase chain reaction**.

4. The method of claim 1 wherein said amplifying step comprises ligase chain reaction or strand displacement amplification.

5. The method of claim 1 wherein said major histocompatibility complex antigen induces a graft versus host or host versus graft response.

6. The method of claim 1 wherein said nucleic acid is RNA.

7. The method of claim 1 wherein said nucleic acid encodes an HLA antigen.

8. The method of claim 1 wherein said amplification product is ionized prior to molecular mass determination.

9. The method of claim 1 further comprising the step of isolating nucleic acid encoding said major histocompatibility complex antigen prior to contacting said nucleic acid with said at least one pair of **oligonucleotide primers**.

10. The method of claim 1 further comprising the step of performing steps a)-d) using a different **oligonucleotide primer** pair and comparing the results to one or more molecular mass amplification products obtained by performing steps a)-c) on a different plurality of known organisms from those in step d).

contained in a database of molecular masses.

12. The method of claim 1 wherein said amplification product is ionized by electrospray ionization, matrix-assisted laser desorption or fast atom bombardment.

13. The method of claim 1 wherein said molecular mass is determined by mass spectrometry.

14. The method of claim 11 wherein said mass spectrometry is selected from the group consisting of Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS), ion trap, quadrupole, magnetic sector, time of flight (TOF), Q-TOF and triple quadrupole.

15. The method of claim 1 further comprising performing step b) in the presence of an analog of adenine, thymidine, guanosine or cytidine having a different molecular weight than adenosine, thymidine, guanosine or cytidine.

16. The method of claim 1 wherein said **oligonucleotide primer** comprises a base analog at positions 1 and 2 of each triplet within said **primer**, wherein said base analog binds with increased affinity to its complement compared to the native base.

17. The method of claim 16 wherein said **primer** comprises a universal base at position 3 of each triplet within said **primer**.

18. The method of claim 16 wherein said base analog is selected from the group consisting of 2,6-diaminopurine, propyne T, propyne G, phenoxazines and G-clamp.

19. The method of claim 16 wherein said universal base is selected from the group consisting of inosine, guanine uridine, 5-nitroindole, 3-nitropyrrole, dP, dK, and 1-(2-deoxy- β -D-ribofuranosyl)-imidazole-4-carboxamide.

20. A method of identifying a major histocompatibility complex antigen in an organ recipient or donor organ comprising: a) contacting nucleic acid encoding said major histocompatibility complex antigen in said organ recipient or donor organ with at least one pair of **oligonucleotide primers** which hybridize to sequences of said nucleic acid, wherein said sequences flank a variable nucleic acid sequence; b) amplifying said variable nucleic acid sequence to produce an amplification product; c) determining the base composition of said amplification product; and d) comparing said base composition to one or more base compositions of amplification products obtained by performing steps a)-c) on a plurality of known organisms, wherein a match identifies said major histocompatibility complex antigen in said organ recipient or donor organ.

21. The method of claim 20 wherein said sequences to which said at least one pair of **oligonucleotide primers** hybridize are highly conserved.

22. The method of claim 20 wherein said amplifying step comprises **polymerase chain reaction**.

23. The method of claim 20 wherein said amplifying step comprises ligase chain reaction or strand displacement amplification.

24. The method of claim 20 wherein said major histocompatibility complex antigen induces a host versus graft or graft versus host response.

25. The method of claim 20 wherein said nucleic acid is RNA.

26. The method of claim 20 wherein said nucleic acid encodes an HLA antigen.

27. The method of claim 20 wherein said amplification product is ionized prior to base composition determination.

28. The method of claim 20 further comprising the step of isolating nucleic acid encoding said major histocompatibility complex antigen prior to contacting said nucleic acid with said at least one pair of **oligonucleotide primers**.

29. The method of claim 20 further comprising the step of performing steps a)-d) using a different **oligonucleotide primer** pair and comparing the results to one or more base compositions of amplification product obtained by performing steps a)-c) on a different plurality of known organisms from those in step d).

30. The method of claim 20 wherein said one or more base composition

31. The method of claim 20 wherein said amplification product is ionized by electrospray ionization, matrix-assisted laser desorption or fast atom bombardment.

32. The method of claim 20 wherein said base composition signature is determined by mass spectrometry.

33. The method of claim 32 wherein said mass spectrometry is selected from the group consisting of Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS), ion trap, quadrupole, magnetic sector, time of flight (TOF), q-TOF and triple quadrupole.

34. The method of claim 20 further comprising performing step b) in the presence of an analog of adenine, thymidine, guanosine or cytidine having a different molecular weight than adenosine, thymidine, guanosine or cytidine.

35. The method of claim 20 wherein said **oligonucleotide primer** comprises a base analog at positions 1 and 2 of each triplet within said **primer**, wherein said base analog binds with increased affinity to its complement compared to the native base.

36. The method of claim 35 wherein said **primer** comprises a universal base at position 3 of each triplet within said **primer**.

37. The method of claim 35 wherein said base analog is selected from the group consisting of 2,6-diaminopurine, propyne T, propyne G, phenoxazines and G-clamp.

38. The method of claim 35 wherein said universal base is selected from the group consisting of inosine, guanidine uridine, 5-nitroindole, 3-nitropyrrole, dP, dK, and 1-(2-deoxy- β -D-ribofuranosyl)-imidazole-4-carboxamide.

39. A method of determining the absence of a major histocompatibility complex antigen in an organ recipient or donor organ comprising: a) contacting said organ recipient or donor organ sample suspected of containing nucleic acid encoding said major histocompatibility complex antigen with at least one pair of **oligonucleotide primers** which are capable of hybridizing to sequences of said nucleic acid, wherein said sequences flank a variable nucleic acid sequence of said major histocompatibility complex antigen; b) treating said variable nucleic acid sequence under amplification conditions capable of producing an amplification product of said variable nucleic acid sequence; c) performing spectroscopy to determine the molecular mass or base composition of all amplification products; and d) comparing said molecular mass to one or more molecular masses of amplification products or said base composition to one or more base compositions of amplification products obtained by performing steps a)-c) on a plurality of known organisms, wherein the lack of a match indicates that said major histocompatibility complex antigen is absent from said organ recipient or donor organ sample.

40. The method of claim 39 further comprising determining the presence of a positive control.

41. The method of claim 40 wherein said positive control is a known major histocompatibility complex antigen or residual **primer** signal.

L5 ANSWER 15 OF 112 USPATFULL on STN

2004:158530 Secondary structure defining database and methods for determining identity and geographic origin of an unknown bioagent in blood, bodily fluids, and bodily tissues thereby.

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US 2004121329 A1 20040624

APPLICATION: US 2002-323211 A1 20021218 (10)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates generally to the field of investigational bioinformatics and more particularly to secondary structure defining databases. The present invention further relates to methods for interrogating a database as a source of molecular masses of known bioagents for comparing against the molecular mass of an unknown or selected bioagent to determine either the identity of the selected bioagent, and/or to determine the origin of the selected bioagent. The

CLM

course of treatment and/or irradiation of the bioagent in such cases as biological warfare. Furthermore, the determination of the geographic origin of a selected bioagent will facilitate the identification of potential criminal identity.

What is claimed is:

1. A method of identifying an unknown bioagent in an animal sample using a database of molecular masses of known bioagents comprising: contacting nucleic acid from said bioagent in said animal sample with at least one pair of **oligonucleotide primers** that hybridize to.. sequences of said nucleic acid, wherein said sequences flank a variable nucleic acid sequence of said bioagent in said animal sample; producing an amplification product of said variable nucleic acid sequence; determining a first molecular mass of said amplification product; and comparing said first molecular mass to the molecular masses of known bioagents, thereby identifying the unknown bioagent in said animal sample.

2. The method of claim 1 wherein said sequences to which said at least one pair of **oligonucleotide primers** hybridize are highly conserved.

3. The method of claim 1 wherein said sequences to which said at least one pair of **oligonucleotide primers** hybridize are highly conserved across at least two species.

4. The method of claim 1 further comprising the step of isolating a nucleic acid from said animal sample prior to contacting said nucleic acid with said at least one pair of **oligonucleotide primers**, wherein the comparing step further comprises comparing a base-pair count resulting from a translation of the corresponding molecular mass, and wherein a master database of molecular masses of known bioagents further includes a translation of said molecular masses of known bioagents to corresponding base-pair counts of each known bioagent resulting from a specific **primer** pair set and comparing the base-pair count of said unknown bioagent against the obtained base-pair count of known bioagents for the selected **primer** pair set for determining the identity of said unknown bioagent in said animal sample.

5. The method of claim 4 further comprising the step of reconciling the database of molecular masses of known bioagents with the master database of molecular masses of known bioagents.

6. The method of claim 1 wherein said bioagent is a bacterium, parasite, fungi, virus, cell or spore.

7. The method of claim 1 wherein said animal sample is blood, a bodily fluid, or a bodily tissue.

8. The method of claim 1 wherein said amplification product is ionized by electrospray ionization, matrix assisted laser desorption or fast atom bombardment prior to molecular mass determination.

9. The method of claim 1 wherein said molecular mass is determined by mass spectrometry.

10. The method of claim 5 wherein said master database of molecular masses of known bioagents and the database of molecular masses of known bioagents are reconciled over a network.

11. The method of claim 4 wherein the identity is determined by statistically correlating the molecular mass of the unknown bioagent with at least one molecular mass of said master database.

12. A database having cell-data positional significance comprising at least a first table of a plurality of data-containing cells, said first table organized into at least a first row and a second row, each row having columns and data-containing cells; and wherein said data-containing cells have an alignment with at least one other row for differentiating aligned from non-aligned data-containing cells, and wherein said differentiation in alignment of said data-containing cells designates a structural feature of a polymer present in an animal sample.

13. The database according to claim 12 wherein said alignment is a vertical alignment according to base pair homology along a linear segment within each polymer.

14. The database according to claim 12 wherein said vertical alignment further aligns cell-data according to inter-species conserved regions.

15. The database according to claim 12 wherein the structural feature is a bulge or a loop.

17. The method of claim 12 wherein said sample is blood, a bodily fluid, or a bodily tissue.

18. A service providing information related to a bioagent in an animal sample comprising: providing a dimensional master database for storing a molecular mass, an identity and a detail corresponding to a plurality of known bioagents and, said master database storing the molecular mass, the identity and the detail for a plurality of known bioagents; interrogating the master database with an identification request of an unknown bioagent in said animal sample to generate a response; and delivering said response from the master database to a requester.

19. The service according to claim 18 wherein the molecular mass is of a selected portion of the known bioagent, the identity comprises at least a geographic origin and a name for the known bioagent, and the detail comprises at least a treatment.

20. The service according to claim 18 wherein the request comprises a symptomatology and the identification comprises a recommended pair of **primers** for hybridizing to sequences of nucleic acid flanking a variable nucleic acid sequence of the unknown bioagent, and said pair of **primers** are hybridized to the sequences of nucleic acid flanking a variable nucleic acid sequence of the unknown bioagent in said animal sample.

21. The service according to claim 20 wherein the nucleic acid sequence of the unknown bioagent in said animal sample between said pair of **primers** defines the selected portion of both the known bioagents and the unknown bioagent in said animal sample.

22. The service according to claim 21 wherein the response is delivered through a network.

23. The service according to claim 21 wherein the request comprises a molecular mass of the unknown bioagent for the selected portion and where the response generated thereto comprises a set of molecular masses for analogous selected portions of known bioagents, and said set comprising at least one molecular mass from the master database.

24. The service according to claim 22 wherein the network is a local area network.

25. The service according to claim 22 wherein the network is a wide area network.

26. The service according to claim 23 wherein the network is the internet.

27. A method of determining a geographical origin of a selected bioagent in an animal sample using a database of molecular masses of known bioagents comprising: contacting nucleic acid from said selected bioagent in said animal sample with at least one pair of **oligonucleotide primers** which hybridize to sequences of said nucleic acid, wherein said sequences flank a variable nucleic acid sequence of the bioagent in said animal sample; producing an amplification product of said variable nucleic acid sequence; determining a first molecular mass of said amplification product; and comparing said first molecular mass to the molecular masses of known bioagents for determining a geographic origin of said selected bioagent, said comparison determining an identity and a geographic origin of said selected bioagent in said animal sample.

28. The method of claim 27 wherein said sequences to which said at least one pair of **oligonucleotide primers** hybridize are highly conserved.

29. The method of claim 27 wherein said sequences to which said at least one pair of **oligonucleotide primers** hybridize are highly conserved across species.

30. The method of claim 27 further comprising the step of isolating a nucleic acid from said selected bioagent in said animal sample prior to contacting said nucleic acid with said at least one pair of **oligonucleotide primers**, wherein the comparing step further comprises interrogating a master database of molecular masses of known bioagents for obtaining molecular masses of known bioagents and comparing the molecular mass of said selected bioagent against the obtained molecular masses of known bioagents thereby determining an origin of said selected bioagent in said animal sample.

31. The method of claim 30 further comprising the step of reconciling the database of molecular masses of known bioagents with the master

32. The method of claim 27 wherein said bioagent is a bacterium, parasite, fungi, virus, cell or spore.

33. The method of claim 27 wherein said animal sample is blood, a bodily fluid, or a bodily tissue.

34. The method of claim 27 wherein said amplification product is ionized by electrospray ionization, matrix assisted laser desorption or fast atom bombardment prior to molecular mass determination.

35. The method of claim 27 wherein said molecular mass is determined by mass spectrometry.

36. The method of claim 30 wherein said master database of molecular masses of known bioagents and the database of molecular masses of known bioagents are reconciled over a network.

37. The method of claim 30 wherein the origin comprises a statistical group of matching molecular masses and the geographic origin corresponding thereto.

L5 ANSWER 16 OF 112 USPATFULL on STN

2004:158518 Secondary structure defining database and methods for determining identity and geographic origin of an unknown bioagent in containers thereby

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US 2004121315 A1 20040624

APPLICATION: US 2002-326644 A1 20021218 (10)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates generally to the field of investigational bioinformatics and more particularly to secondary structure defining databases. The present invention further relates to methods for interrogating a database as a source of molecular masses of known bioagents for comparing against the molecular mass of an unknown or selected bioagent to determine either the identity of the selected bioagent, and/or to determine the origin of the selected bioagent. The identification of the bioagent is important for determining a proper course of treatment and/or irradiation of the bioagent in such cases as biological warfare. Furthermore, the determination of the geographic origin of a selected bioagent will facilitate the identification of potential criminal identity.

CLM What is claimed is:

1. A method of identifying an unknown bioagent in a container using a database of molecular masses of known bioagents comprising: contacting nucleic acid from said bioagent in said container with at least one pair of **oligonucleotide primers** that hybridize to sequences of said nucleic acid, wherein said sequences flank a variable nucleic acid sequence of said bioagent in said container; producing an amplification product of said variable nucleic acid sequence; determining a first molecular mass of said amplification product; and comparing said first molecular mass to the molecular masses of known bioagents, thereby identifying the unknown bioagent in said container.

2. The method of claim 1 wherein said sequences to which said at least one pair of **oligonucleotide primers** hybridize are highly conserved.

3. The method of claim 1 wherein said sequences to which said at least one pair of **oligonucleotide primers** hybridize are highly conserved across at least two species.

4. The method of claim 1 further comprising the step of isolating a nucleic acid from said container prior to contacting said nucleic acid with said at least one pair of **oligonucleotide primers**, wherein the comparing step further comprises comparing a base-pair count resulting from a translation of the corresponding molecular mass, and wherein a master database of molecular masses of known bioagents further includes a translation of said molecular masses of known bioagents to corresponding base-pair counts of each known bioagent resulting from a specific **primer** pair set and comparing the base-pair count of said unknown bioagent against the obtained base-pair count of known bioagents for the selected **primer** pair set for determining the identity of said unknown bioagent in said container.

5. The method of claim 4 further comprising the step of reconciling the

of molecular masses of known bioagents.

6. The method of claim 1 wherein said bioagent is a bacterium, parasite, fungi, virus, cell or spore.

7. The method of claim 1 wherein said container is a package, box, envelope, mail tube, or railroad box car.

8. The method of claim 1 wherein said amplification product is ionized by electrospray ionization, matrix assisted laser desorption or fast atom bombardment prior to molecular mass determination.

9. The method of claim 1 wherein said molecular mass is determined by mass spectrometry.

10. The method of claim 5 wherein said master database of molecular masses of known bioagents and the database of molecular masses of known bioagents are reconciled over a network.

11. The method of claim 4 wherein the identity is determined by statistically correlating the molecular mass of the unknown bioagent with at least one molecular mass of said master database.

12. A database having cell-data positional significance comprising at least a first table of a plurality of data-containing cells, said first table organized into at least a first row and a second row, each row having columns and data-containing cells; and wherein said data-containing cells have an alignment with at least one other row for differentiating aligned from non-aligned data-containing cells, and wherein said differentiation in alignment of said data-containing cells designates a structural feature of a polymer present in a container.

13. The database according to claim 12 wherein said alignment is a vertical alignment according to base pair homology along a linear segment within each polymer.

14. The database according to claim 12 wherein said vertical alignment further aligns cell-data according to inter-species conserved regions.

15. The database according to claim 12 wherein the structural feature is a bulge or a loop.

16. The database according to claim 12 wherein the polymer is an RNA.

17. The method of claim 12 wherein said container is a box or envelope.

18. A service providing information related to a bioagent in a container comprising: providing a dimensional master database for storing a molecular mass, an identity and a detail corresponding to a plurality of known bioagents and, said master database storing the molecular mass, the identity and the detail for a plurality of known bioagents; interrogating the master database with an identification request of an unknown bioagent in a container to generate a response; and delivering said response from the master database to a requester.

19. The service according to claim 18 wherein the molecular mass is of a selected portion of the known bioagent, the identity comprises at least a geographic origin and a name for the known bioagent, and the detail comprises at least a treatment.

20. The service according to claim 18 wherein the request comprises a symptomatology and the identification comprises a recommended pair of **primers** for hybridizing to sequences of nucleic acid flanking a variable nucleic acid sequence of the unknown bioagent, and said pair of **primers** are hybridized to the sequences of nucleic acid flanking a variable nucleic acid sequence of the unknown bioagent.

21. The service according to claim 20 wherein the nucleic acid sequence of the unknown bioagent between said pair of **primers** defines the selected portion of both the known bioagents and the unknown bioagent.

22. The service according to claim 21 wherein the response is delivered through a network.

23. The service according to claim 21 wherein the request comprises a molecular mass of the unknown bioagent for the selected portion and where the response generated thereto comprises a set of molecular masses for analogous selected portions of known bioagents, and said set comprising at least one molecular mass from the master database.

24. The service according to claim 22 wherein the network is a local area network.

25. The service according to claim 22 wherein the network is a wide area network.

26. The service according to claim 23 wherein the network is the internet.

27. A method of determining a geographical origin of a selected bioagent in a container using a database of molecular masses of known bioagents comprising: contacting nucleic acid from said selected bioagent in said container with at least one pair of **oligonucleotide primers** which hybridize to sequences of said nucleic acid, wherein said sequences flank a variable nucleic acid sequence of the bioagent in said container; producing an amplification product of said variable nucleic acid sequence; determining a first molecular mass of said amplification product; and comparing said first molecular mass to the molecular masses of known bioagents for determining a geographic origin of said selected bioagent, said comparison determining an identity and a geographic origin of said selected bioagent in said container.

28. The method of claim 27 wherein said sequences to which said at least one pair of **oligonucleotide primers** hybridize are highly conserved.

29. The method of claim 27 wherein said sequences to which said at least one pair of **oligonucleotide primers** hybridize are highly conserved across species.

30. The method of claim 27 further comprising the step of isolating a nucleic acid from said selected bioagent prior to contacting said nucleic acid with said at least one pair of **oligonucleotide primers**, wherein the comparing step further comprises interrogating a master database of molecular masses of known bioagents for obtaining molecular masses of known bioagents and comparing the molecular mass of said selected bioagent against the obtained molecular masses of known bioagents thereby determining an origin of said selected bioagent.

31. The method of claim 30 further comprising the step of reconciling the database of molecular masses of known bioagents with the master database of molecular masses of known bioagents.

32. The method of claim 27 wherein said bioagent is a bacterium, parasite, fungi, virus, cell or spore.

33. The method of claim 27 wherein said container is a package, box, envelope, mail tube, or railroad box car.

34. The method of claim 27 wherein said amplification product is ionized by electrospray ionization, matrix assisted laser desorption or fast atom bombardment prior to molecular mass determination.

35. The method of claim 27 wherein said molecular mass is determined by mass spectrometry.

36. The method of claim 30 wherein said master database of molecular masses of known bioagents and the database of molecular masses of known bioagents are reconciled over a network.

37. The method of claim 30 wherein the origin comprises a statistical group of matching molecular masses and the geographic origin corresponding thereto.

L5 ANSWER 17 OF 112 USPATFULL on STN

2004:158517 Methods for rapid detection and identification of bioagents in containers.

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US 2004121314 A1 20040624

APPLICATION: US 2002-326642 A1 20021218 (10)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Method for detecting and identifying unknown bioagents, including bacteria, viruses and the like, by a combination of nucleic acid amplification and molecular weight determination using primers which hybridize to conserved sequence regions of nucleic acids derived from a bioagent and which bracket variable sequence regions that uniquely identify the bioagent. The result is a "base composition signature" (BCS) which is then matched against a database of base composition signatures, by which the bioagent is identified.

CLM What is claimed is:

comprising: a) contacting nucleic acid from said bioagent in said container with at least one pair of **oligonucleotide primers** which hybridize to sequences of said nucleic acid, wherein said sequences flank a variable nucleic acid sequence of the bioagent; b) amplifying said variable nucleic acid sequence to produce an amplification product; c) determining the molecular mass of said amplification product; and d) comparing said molecular mass to one or more molecular masses of amplification products obtained by performing steps a)-c) on a plurality of known organisms, wherein a match identifies said unknown bioagent in said container.

2. The method of claim 1 wherein said sequences to which said at least one pair of **oligonucleotide primers** hybridize are highly conserved.

3. The method of claim 1 wherein said amplifying step comprises **polymerase chain reaction**.

4. The method of claim 1 wherein said amplifying step comprises ligase chain reaction or strand displacement amplification.

5. The method of claim 1 wherein said bioagent is a bacterium, virus, parasite, fungi, cell or spore.

6. The method of claim 1 wherein said nucleic acid is ribosomal RNA.

7. The method of claim 1 wherein said nucleic acid encodes RNase P or an RNA-dependent RNA polymerase.

8. The method of claim 1 wherein said amplification product is ionized prior to molecular mass determination.

9. The method of claim 1 further comprising the step of isolating nucleic acid from said bioagent prior to contacting said nucleic acid with said at least one pair of **oligonucleotide primers**.

10. The method of claim 1 further comprising the step of performing steps a)-d) using a different **oligonucleotide primer** pair and comparing the results to one or more molecular mass amplification products obtained by performing steps a)-c) on a different plurality of known organisms from those in step d).

11. The method of claim 1 wherein said one or more molecular masses are contained in a database of molecular masses.

12. The method of claim 1 wherein said amplification product is ionized by electrospray ionization, matrix-assisted laser desorption or fast atom bombardment.

13. The method of claim 1 wherein said molecular mass is determined by mass spectrometry.

14. The method of claim 11 wherein said mass spectrometry is selected from the group consisting of Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS), ion trap, quadrupole, magnetic sector, time of flight (TOF), Q-TOF and triple quadrupole.

15. The method of claim 1 further comprising performing step b) in the presence of an analog of adenine, thymidine, guanosine or cytidine having a different molecular weight than adenosine, thymidine, guanosine or cytidine.

16. The method of claim 1 wherein said **oligonucleotide primer** comprises a base analog at positions 1 and 2 of each triplet within said **primer**, wherein said base analog binds with increased affinity to its complement compared to the native base.

17. The method of claim 16 wherein said **primer** comprises a universal base at position 3 of each triplet within said **primer**.

18. The method of claim 16 wherein said base analog is selected from the group consisting of 2,6-diaminopurine, propyne T, propyne G, phenoxazines and G-clamp.

19. The method of claim 16 wherein said universal base is selected from the group consisting of inosine, guanidine uridine, 5-nitroindole, 3-nitropyrrole, dP, dK, and 1-(2-deoxy- β -D-ribofuranosyl)-imidazole-4-carboxamide.

20. The method of claim 1 wherein said container is a package, box, envelope, mail tube, or railroad box car.

21. A method of identifying an unknown bioagent in a container

container with at least one pair of **oligonucleotide primers** which hybridize to sequences of said nucleic acid, wherein said sequences flank a variable nucleic acid sequence; b) amplifying said variable nucleic acid sequence to produce an amplification product; c) determining the base composition of said amplification product; and d) comparing said base composition to one or more base compositions of amplification products obtained by performing steps a)-c) on a plurality of known organisms, wherein a match identifies said unknown bioagent in said container.

22. The method of claim 21 wherein said sequences to which said at least one pair of **oligonucleotide primers** hybridize are highly conserved.

23. The method of claim 21 wherein said amplifying step comprises **polymerase chain reaction**.

24. The method of claim 21 wherein said amplifying step comprises ligase chain reaction or strand displacement amplification.

25. The method of claim 21 wherein said bioagent is a bacterium, virus, fungi, parasite, cell or spore.

26. The method of claim 21 wherein said nucleic acid is ribosomal RNA.

27. The method of claim 21 wherein said nucleic acid encodes RNase P or an RNA-dependent RNA polymerase.

28. The method of claim 21 wherein said amplification product is ionized prior to base composition determination.

29. The method of claim 21 further comprising the step of isolating nucleic acid from said bioagent prior to contacting said nucleic acid with said at least one pair of **oligonucleotide primers**.

30. The method of claim 21 further comprising the step of performing steps a)-d) using a different **oligonucleotide primer** pair and comparing the results to one or more base compositions of amplification product obtained by performing steps a)-c) on a different plurality of known organisms from those in step d).

31. The method of claim 21 wherein said one or more base composition signatures are contained in a database of base composition signatures.

32. The method of claim 21 wherein said amplification product is ionized by electrospray ionization, matrix-assisted laser desorption or fast atom bombardment.

33. The method of claim 21 wherein said base composition signature is determined by mass spectrometry.

34. The method of claim 33 wherein said mass spectrometry is selected from the group consisting of Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS), ion trap, quadrupole, magnetic sector, time of flight (TOF), q-TOF and triple quadrupole.

35. The method of claim 21 further comprising performing step b) in the presence of an analog of adenine, thymidine, guanosine or cytidine having a different molecular weight than adenosine, thymidine, guanosine or cytidine.

36. The method of claim 21 wherein said **oligonucleotide primer** comprises a base analog at positions 1 and 2 of each triplet within said **primer**, wherein said base analog binds with increased affinity to its complement compared to the native base.

37. The method of claim 36 wherein said **primer** comprises a universal base at position 3 of each triplet within said **primer**.

38. The method of claim 36 wherein said base analog is selected from the group consisting of 2,6-diaminopurine, propyne T, propyne G, phenoxazines and G-clamp.

39. The method of claim 36 wherein said universal base is selected from the group consisting of inosine, guanidine uridine, 5-nitroindole, 3-nitropyrrole, dP, dK, and 1-(2-deoxy- β -D-ribofuranosyl)-imidazole-4-carboxamide.

40. The method of claim 21 wherein said container is a package, box, envelope, mail tube, or railroad box car.

41. A method of determining the absence of a bioagent in a container sample comprising: a) contacting said container sample suspected of

oligonucleotide primers which are capable of hybridizing to sequences of said nucleic acid, wherein said sequences flank a variable nucleic acid sequence of said bioagent; b) treating said variable nucleic acid sequence under amplification conditions capable of producing an amplification product of said variable nucleic acid sequence; c) performing spectroscopy to determine the molecular mass or base composition of all amplification products; and d) comparing said molecular mass to one or more molecular masses of amplification products or said base composition to one or more base compositions of amplification products obtained by performing steps a)-c) on a plurality of known organisms, wherein the lack of a match indicates that said bioagent is absent from said container sample.

42. The method of claim 41 further comprising determining the presence of a positive control.

43. The method of claim 42 wherein said positive control is a known bioagent or residual **primer** signal.

L5 ANSWER 18 OF 112 USPTAFULL on STN

2004:158516 Methods for rapid detection and identification of bioagents in organs for transplantation.

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US 2004121313 A1 20040624

APPLICATION: US 2002-326051 A1 20021218 (10)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Method for detecting and identifying unknown bioagents, including bacteria, viruses and the like, by a combination of nucleic acid amplification and molecular weight determination using primers which hybridize to conserved sequence regions of nucleic acids derived from a bioagent and which bracket variable sequence regions that uniquely identify the bioagent. The result is a "base composition signature" (BCS) which is then matched against a database of base composition signatures, by which the bioagent is identified.

CLM What is claimed is:

1. A method of identifying an unknown bioagent in a donor organ sample comprising: a) contacting nucleic acid from said bioagent in said donor organ sample with at least one pair of **oligonucleotide primers** which hybridize to sequences of said nucleic acid, wherein said sequences flank a variable nucleic acid sequence of the bioagent; b) amplifying said variable nucleic acid sequence to produce an amplification product; c) determining the molecular mass of said amplification product; and d) comparing said molecular mass to one or more molecular masses of amplification products obtained by performing steps a)-c) on a plurality of known organisms, wherein a match identifies said unknown bioagent in said donor organ sample.

2. The method of claim 1 wherein said sequences to which said at least one pair of **oligonucleotide primers** hybridize are highly conserved.

3. The method of claim 1 wherein said amplifying step comprises **polymerase chain reaction**.

4. The method of claim 1 wherein said amplifying step comprises ligase chain reaction or strand displacement amplification.

5. The method of claim 1 wherein said bioagent is a bacterium, virus, parasite, fungi, cell or spore.

6. The method of claim 1 wherein said nucleic acid is ribosomal RNA.

7. The method of claim 1 wherein said nucleic acid encodes RNase P or an RNA-dependent RNA polymerase.

8. The method of claim 1 wherein said amplification product is ionized prior to molecular mass determination.

9. The method of claim 1 further comprising the step of isolating nucleic acid from said bioagent prior to contacting said nucleic acid with said at least one pair of **oligonucleotide primers**.

10. The method of claim 1 further comprising the step of performing steps a)-d) using a different **oligonucleotide primer** pair and comparing the results to one or more molecular mass amplification products obtained by performing steps a)-c) on a different plurality of known organisms from those in step d).

11. The method of claim 1 wherein said one or more molecular masses are contained in a database of molecular masses.
12. The method of claim 1 wherein said amplification product is ionized by electrospray ionization, matrix-assisted laser desorption or fast atom bombardment.
13. The method of claim 1 wherein said molecular mass is determined by mass spectrometry.
14. The method of claim 11 wherein said mass spectrometry is selected from the group consisting of Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS), ion trap, quadrupole, magnetic sector, time of flight (TOF), Q-TOF and triple quadrupole.
15. The method of claim 1 further comprising performing step b) in the presence of an analog of adenine, thymidine, guanosine or cytidine having a different molecular weight than adenosine, thymidine, guanosine or cytidine.
16. The method of claim 1 wherein said **oligonucleotide primer** comprises a base analog at positions 1 and 2 of each triplet within said **primer**, wherein said base analog binds with increased affinity to its complement compared to the native base.
17. The method of claim 16 wherein said **primer** comprises a universal base at position 3 of each triplet within said **primer**.
18. The method of claim 16 wherein said base analog is selected from the group consisting of 2,6-diaminopurine, propyne T, propyne G, phenoxazines and G-clamp.
19. The method of claim 16 wherein said universal base is selected from the group consisting of inosine, guanidine uridine, 5-nitroindole, 3-nitropyrrole, dP, dK, and 1-(2-deoxy- β -D-ribofuranosyl)-imidazole-4-carboxamide.
20. The method of claim 5 wherein said virus is West Nile virus, a hepatitis virus, or human immunodeficiency virus.
21. A method of identifying an unknown bioagent in a donor organ sample comprising: a) contacting nucleic acid from said bioagent in said donor organ sample with at least one pair of **oligonucleotide primers** which hybridize to sequences of said nucleic acid, wherein said sequences flank a variable nucleic acid sequence; b) amplifying said variable nucleic acid sequence to produce an amplification product; c) determining the base composition of said amplification product; and d) comparing said base composition to one or more base compositions of amplification products obtained by performing steps a)-c) on a plurality of known organisms, wherein a match identifies said unknown bioagent in said donor organ sample.
22. The method of claim 21 wherein said sequences to which said at least one pair of **oligonucleotide primers** hybridize are highly conserved.
23. The method of claim 21 wherein said amplifying step comprises **polymerase chain reaction**.
24. The method of claim 21 wherein said amplifying step comprises ligase chain reaction or strand displacement amplification.
25. The method of claim 21 wherein said bioagent is a bacterium, virus, fungi, parasite, cell or spore.
26. The method of claim 21 wherein said nucleic acid is ribosomal RNA.
27. The method of claim 21 wherein said nucleic acid encodes RNase P or an RNA-dependent RNA polymerase.
28. The method of claim 21 wherein said amplification product is ionized prior to base composition determination.
29. The method of claim 21 further comprising the step of isolating nucleic acid from said bioagent prior to contacting said nucleic acid with said at least one pair of **oligonucleotide primers**.
30. The method of claim 21 further comprising the step of performing steps a)-d) using a different **oligonucleotide primer** pair and comparing the results to one or more base compositions of amplification product obtained by performing steps a)-c) on a different plurality of known organisms from those in step d).

signatures are contained in a database of base composition signatures.

32. The method of claim 21 wherein said amplification product is ionized by electrospray ionization, matrix-assisted laser desorption or fast atom bombardment.

33. The method of claim 21 wherein said base composition signature is determined by mass spectrometry.

34. The method of claim 33 wherein said mass spectrometry is selected from the group consisting of Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS), ion trap, quadrupole, magnetic sector, time of flight (TOF), q-TOF and triple quadrupole.

35. The method of claim 21 further comprising performing step b) in the presence of an analog of adenine, thymidine, guanosine or cytidine having a different molecular weight than adenosine, thymidine, guanosine or cytidine.

36. The method of claim 21 wherein said **oligonucleotide primer** comprises a base analog at positions 1 and 2 of each triplet within said **primer**, wherein said base analog binds with increased affinity to its complement compared to the native base.

37. The method of claim 36 wherein said **primer** comprises a universal base at position 3 of each triplet within said **primer**.

38. The method of claim 36 wherein said base analog is selected from the group consisting of 2,6-diaminopurine, propyne T, propyne G, phenoxazines and G-clamp.

39. The method of claim 36 wherein said universal base is selected from the group consisting of inosine, guanidine uridine, 5-nitroindole, 3-nitropyrrole, dP, dK, and 1-(2-deoxy- β -D-ribofuranosyl)-imidazole-4-carboxamide.

40. The method of claim 25 wherein said virus is West Nile virus, a hepatitis virus, or human immunodeficiency virus.

41. A method of determining the absence of a bioagent in a donor organ sample comprising: a) contacting said donor organ sample suspected of containing nucleic acid encoding said bioagent with at least one pair of **oligonucleotide primers** which are capable of hybridizing to sequences of said nucleic acid, wherein said sequences flank a variable nucleic acid sequence of said bioagent; b) treating said variable nucleic acid sequence under amplification conditions capable of producing an amplification product of said variable nucleic acid sequence; c) performing spectroscopy to determine the molecular mass or base composition of all amplification products; and d) comparing said molecular mass to one or more molecular masses of amplification products or said base composition to one or more base compositions of amplification products obtained by performing steps a)-c) on a plurality of known organisms, wherein the lack of a match indicates that said bioagent is absent from said donor organ sample.

42. The method of claim 41 further comprising determining the presence of a positive control.

43. The method of claim 42 wherein said positive control is a known bioagent or residual **primer** signal.

L5 ANSWER 19 OF 112 USPATFULL ON STN

2004:158515 Methods for rapid detection and identification of the absence of bioagents.

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US 2004121312 A1 20040624

APPLICATION: US 2002-326050 A1 20021218 (10)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Method for detecting and identifying unknown bioagents, including bacteria, viruses and the like, by a combination of nucleic acid amplification and molecular weight determination using primers which hybridize to conserved sequence regions of nucleic acids derived from a bioagent and which bracket variable sequence regions that uniquely identify the bioagent. The result is a "base composition signature" (BCS) which is then matched against a database of base composition signatures, by which the bioagent is identified.

1. A method of determining the absence of a bioagent in a sample comprising: a) contacting said sample suspected of containing nucleic acid encoding said bioagent with at least one pair of **oligonucleotide primers** which are capable of hybridizing to sequences of said nucleic acid, wherein said sequences flank a variable nucleic acid sequence of said bioagent; b) treating said variable nucleic acid sequence under amplification conditions capable of producing an amplification product of said variable nucleic acid sequence; c) performing spectroscopy to determine the molecular mass or base composition of all amplification products; and d) comparing said molecular mass to one or more molecular masses of amplification products or said base composition to one or more base compositions of amplification products obtained by performing steps a)-c) on a plurality of known organisms, wherein the lack of a match indicates that said bioagent is absent from said sample.
2. The method of claim 1 wherein said sequences to which said at least one pair of **oligonucleotide primers** hybridize are highly conserved.
3. The method of claim 1 wherein said amplification conditions comprises **polymerase chain reaction**.
4. The method of claim 1 wherein said amplification conditions comprises ligase chain reaction or strand displacement amplification.
5. The method of claim 1 wherein said bioagent is a bacterium, virus, parasite, fungi, cell or spore.
6. The method of claim 1 wherein said nucleic acid is ribosomal RNA.
7. The method of claim 1 wherein said nucleic acid encodes RNase P or an RNA-dependent RNA polymerase.
8. The method of claim 1 wherein said amplification product is ionized prior to molecular mass determination.
9. The method of claim 1 further comprising the step of isolating nucleic acid from said sample prior to contacting said nucleic acid with said at least one pair of **oligonucleotide primers**.
10. The method of claim 1 further comprising the step of performing steps a)-d) using a different **oligonucleotide primer** pair and comparing the results to one or more molecular mass amplification products obtained by performing steps a)-c) on a different plurality of known organisms from those in step d).
11. The method of claim 1 wherein said one or more molecular masses are contained in a database of molecular masses.
12. The method of claim 1 wherein said spectroscopy comprises electrospray ionization, matrix-assisted laser desorption or fast atom bombardment.
13. The method of claim 1 wherein said molecular mass is determined by mass spectrometry.
14. The method of claim 13 wherein said mass spectrometry is selected from the group consisting of Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS), ion trap, quadrupole, magnetic sector, time of flight (TOF), Q-TOF and triple quadrupole.
15. The method of claim 1 further comprising performing step b) in the presence of an analog of adenine, thymidine, guanosine or cytidine having a different molecular weight than adenosine, thymidine, guanosine or cytidine.
16. The method of claim 1 wherein said **oligonucleotide primer** comprises a base analog at positions 1 and 2 of each triplet within said **primer**, wherein said base analog binds with increased affinity to its complement compared to the native base.
17. The method of claim 16 wherein said **primer** comprises a universal base at position 3 of each triplet within said **primer**.
18. The method of claim 16 wherein said base analog is selected from the group consisting of 2,6-diaminopurine, propyne T, propyne G, phenoxazines and G-clamp.
19. The method of claim 16 wherein said universal base is selected from the group consisting of inosine, guanidine uridine, 5-nitroindole, 3-nitropyrrole, dP, dK, and 1-(2-deoxy- β -D-ribofuranosyl)-imidazole-4-carboxamide.

a positive control.

21. The method of claim 20 wherein said positive control is a known bioagent, internal probe, or residual **primer** signal.

L5 ANSWER 20 OF 112 USPTAFULL on STN

2004:158514 Methods for rapid detection and identification of bioagents in livestock.

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US 2004121311 A1 20040624

APPLICATION: US 2002-325526 A1 20021218 (10)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Method for detecting and identifying unknown bioagents, including bacteria, viruses and the like, by a combination of nucleic acid amplification and molecular weight determination using primers which hybridize to conserved sequence regions of nucleic acids derived from a bioagent and which bracket variable sequence regions that uniquely identify the bioagent. The result is a "base composition signature" (BCS) which is then matched against a database of base composition signatures, by which the bioagent is identified.

CLM What is claimed is:

1. A method of identifying an unknown bioagent in a livestock sample comprising: a) contacting nucleic acid from said bioagent in said livestock sample with at least one pair of **oligonucleotide primers** which hybridize to sequences of said nucleic acid, wherein said sequences flank a variable nucleic acid sequence of the bioagent; b) amplifying said variable nucleic acid sequence to produce an amplification product; c) determining the molecular mass of said amplification product; and d) comparing said molecular mass to one or more molecular masses of amplification products obtained by performing steps a)-c) on a plurality of known organisms, wherein a match identifies said unknown bioagent in said livestock sample.
2. The method of claim 1 wherein said sequences to which said at least one pair of **oligonucleotide primers** hybridize are highly conserved.
3. The method of claim 1 wherein said amplifying step comprises **polymerase chain reaction**.
4. The method of claim 1 wherein said amplifying step comprises ligase chain reaction or strand displacement amplification.
5. The method of claim 1 wherein said bioagent is a bacterium, virus, parasite, fungi, cell or spore.
6. The method of claim 1 wherein said nucleic acid is ribosomal RNA.
7. The method of claim 1 wherein said nucleic acid encodes RNase P or an RNA-dependent RNA polymerase.
8. The method of claim 1 wherein said amplification product is ionized prior to molecular mass determination.
9. The method of claim 1 further comprising the step of isolating nucleic acid from said bioagent prior to contacting said nucleic acid with said at least one pair of **oligonucleotide primers**.
10. The method of claim 1 further comprising the step of performing steps a)-d) using a different **oligonucleotide primer** pair and comparing the results to one or more molecular mass amplification products obtained by performing steps a)-c) on a different plurality of known organisms from those in step d).
11. The method of claim 1 wherein said one or more molecular masses are contained in a database of molecular masses.
12. The method of claim 1 wherein said amplification product is ionized by electrospray ionization, matrix-assisted laser desorption or fast atom bombardment.
13. The method of claim 1 wherein said molecular mass is determined by mass spectrometry.
14. The method of claim 11 wherein said mass spectrometry is selected from the group consisting of Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS), ion trap, quadrupole, magnetic sector,

15. The method of claim 1 further comprising performing step b) in the presence of an analog of adenine, thymidine, guanosine or cytidine having a different molecular weight than adenosine, thymidine, guanosine or cytidine.

16. The method of claim 1 wherein said **oligonucleotide primer** comprises a base analog at positions 1 and 2 of each triplet within said **primer**, wherein said base analog binds with increased affinity to its complement compared to the native base.

17. The method of claim 16 wherein said **primer** comprises a universal base at position 3 of each triplet within said **primer**.

18. The method of claim 16 wherein said base analog is selected from the group consisting of 2,6-diaminopurine, propyne T, propyne G, phenoxazines and G-clamp.

19. The method of claim 16 wherein said universal base is selected from the group consisting of inosine, guanidine uridine, 5-nitroindole, 3-nitropyrrole, dP, dK, and 1-(2-deoxy- β -D-ribofuranosyl)-imidazole-4-carboxamide.

20. The method of claim 1 wherein said bioagent causes mad cow disease or hoof and mouth disease.

21. A method of identifying an unknown bioagent in a livestock sample comprising: a) contacting nucleic acid from said bioagent in a livestock sample with at least one pair of **oligonucleotide primers** which hybridize to sequences of said nucleic acid, wherein said sequences flank a variable nucleic acid sequence; b) amplifying said variable nucleic acid sequence to produce an amplification product; c) determining the base composition of said amplification product; and d) comparing said base composition to one or more base compositions of amplification products obtained by performing steps a)-c) on a plurality of known organisms, wherein a match identifies said unknown bioagent in said livestock sample.

22. The method of claim 21 wherein said sequences to which said at least one pair of **oligonucleotide primers** hybridize are highly conserved.

23. The method of claim 21 wherein said amplifying step comprises **polymerase chain reaction**.

24. The method of claim 21 wherein said amplifying step comprises ligase chain reaction or strand displacement amplification.

25. The method of claim 21 wherein said bioagent is a bacterium, virus, fungi, parasite, cell or spore.

26. The method of claim 21 wherein said nucleic acid is ribosomal RNA.

27. The method of claim 21 wherein said nucleic acid encodes RNase P or an RNA-dependent RNA polymerase.

28. The method of claim 21 wherein said amplification product is ionized prior to base composition determination.

29. The method of claim 21 further comprising the step of isolating nucleic acid from said bioagent prior to contacting said nucleic acid with said at least one pair of **oligonucleotide primers**.

30. The method of claim 21 further comprising the step of performing steps a)-d) using a different **oligonucleotide primer** pair and comparing the results to one or more base compositions of amplification product obtained by performing steps a)-c) on a different plurality of known organisms from those in step d).

31. The method of claim 21 wherein said one or more base composition signatures are contained in a database of base composition signatures.

32. The method of claim 21 wherein said amplification product is ionized by electrospray ionization, matrix-assisted laser desorption or fast atom bombardment.

33. The method of claim 21 wherein said base composition signature is determined by mass spectrometry.

34. The method of claim 33 wherein said mass spectrometry is selected from the group consisting of Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS), ion trap, quadrupole, magnetic sector, time of flight (TOF), q-TOF and triple quadrupole.

35. The method of claim 21 further comprising performing step b) in the presence of an analog of adenine, thymidine, guanosine or cytidine having a different molecular weight than adenosine, thymidine, guanosine or cytidine.

36. The method of claim 21 wherein said **oligonucleotide primer** comprises a base analog at positions 1 and 2 of each triplet within said **primer**, wherein said base analog binds with increased affinity to its complement compared to the native base. -

37. The method of claim 36 wherein said **primer** comprises a universal base at position 3 of each triplet within said **primer**.

38. The method of claim 36 wherein said base analog is selected from the group consisting of 2,6-diaminopurine, propyne T, propyne G, phenoxazines and G-clamp.

39. The method of claim 36 wherein said universal base is selected from the group consisting of inosine, guanidine uridine, 5-nitroindole, 3-nitropyrrole, dP, dK, and 1-(2-deoxy- β -D-ribofuranosyl)-imidazole-4-carboxamide.

40. The method of claim 21 wherein said bioagent causes mad cow disease or hoof and mouth disease.

41. A method of determining the absence of a bioagent in a livestock sample comprising: a) contacting said livestock sample suspected of containing nucleic acid encoding said bioagent with at least one pair of **oligonucleotide primers** which are capable of hybridizing to sequences of said nucleic acid, wherein said sequences flank a variable nucleic acid sequence of said bioagent; b) treating said variable nucleic acid sequence under amplification conditions capable of producing an amplification product of said variable nucleic acid sequence; c) performing spectroscopy to determine the molecular mass or base composition of all amplification products; and d) comparing said molecular mass to one or more molecular masses of amplification products or said base composition to one or more base compositions of amplification products obtained by performing steps a)-c) on a plurality of known organisms, wherein the lack of a match indicates that said bioagent is absent from said livestock sample.

42. The method of claim 41 further comprising determining the presence of a positive control.

43. The method of claim 42 wherein said positive control is a known bioagent or residual **primer** signal.

L5 ANSWER 21 OF 112 USPATFULL on STN

2004:158513 Methods for rapid detection and identification of bioagents in forensic studies.

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US 2004121310 A1 20040624

APPLICATION: US 2002-323438 A1 20021218 (10)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Method for detecting and identifying unknown bioagents, including bacteria, viruses and the like, by a combination of nucleic acid amplification and molecular weight determination using primers which hybridize to conserved sequence regions of nucleic acids derived from a bioagent and which bracket variable sequence regions that uniquely identify the bioagent. The result is a "base composition signature" (BCS) which is then matched against a database of base composition signatures, by which the bioagent is identified.

CLM What is claimed is:

1. A method of identifying a geographic origin of an unknown bioagent in a sample comprising: a) contacting nucleic acid from said bioagent with at least one pair of **oligonucleotide primers** which hybridize to sequences of said nucleic acid, wherein said sequences flank a variable nucleic acid sequence of the bioagent; b) amplifying said variable nucleic acid sequence to produce an amplification product; c) determining the molecular mass of said amplification product; and d) comparing said molecular mass to one or more molecular masses of amplification products obtained by performing steps a)-c) on a plurality of known organisms with known geographic origins, wherein a match identifies said geographic origin of said unknown bioagent.

2. The method of claim 1 wherein said sequences to which said at least

3. The method of claim 1 wherein said amplifying step comprises **polymerase chain reaction**.
4. The method of claim 1 wherein said amplifying step comprises ligase chain reaction or strand displacement amplification.
5. The method of claim 1 wherein said bioagent is a bacterium, virus, parasite, fungi, cell or spore.
6. The method of claim 1 wherein said nucleic acid is ribosomal RNA.
7. The method of claim 1 wherein said nucleic acid encodes RNase P or an RNA-dependent RNA polymerase.
8. The method of claim 1 wherein said amplification product is ionized prior to molecular mass determination.
9. The method of claim 1 further comprising the step of isolating nucleic acid from said bioagent prior to contacting said nucleic acid with said at least one pair of **oligonucleotide primers**.
10. The method of claim 1 further comprising the step of performing steps a)-d) using a different **oligonucleotide primer** pair and comparing the results to one or more molecular mass amplification products obtained by performing steps a)-c) on a different plurality of known organisms from those in step d).
11. The method of claim 1 wherein said one or more molecular masses are contained in a database of molecular masses.
12. The method of claim 1 wherein said amplification product is ionized by electrospray ionization, matrix-assisted laser desorption or fast atom bombardment.
13. The method of claim 1 wherein said molecular mass is determined by mass spectrometry.
14. The method of claim 11 wherein said mass spectrometry is selected from the group consisting of Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS), ion trap, quadrupole, magnetic sector, time of flight (TOF), Q-TOF and triple quadrupole.
15. The method of claim 1 further comprising performing step b) in the presence of an analog of adenine, thymidine, guanosine or cytidine having a different molecular weight than adenosine, thymidine, guanosine or cytidine.
16. The method of claim 1 wherein said **oligonucleotide primer** comprises a base analog at positions 1 and 2 of each triplet within said **primer**, wherein said base analog binds with increased affinity to its complement compared to the native base.
17. The method of claim 16 wherein said **primer** comprises a universal base at position 3 of each triplet within said **primer**.
18. The method of claim 16 wherein said base analog is selected from the group consisting of 2,6-diaminopurine, propyne T, propyne G, phenoxazines and G-clamp.
19. The method of claim 16 wherein said universal base is selected from the group consisting of inosine, guanidine uridine, 5-nitroindole, 3-nitropyrrole, dP, dK, and 1-(2-deoxy- β -D-ribofuranosyl)-imidazole-4-carboxamide.
20. A method of identifying a geographic origin of an unknown bioagent in a sample comprising: a) contacting nucleic acid from said bioagent with at least one pair of **oligonucleotide primers** which hybridize to sequences of said nucleic acid, wherein said sequences flank a variable nucleic acid sequence; b) amplifying said variable nucleic acid sequence to produce an amplification product; c) determining the base composition of said amplification product; and d) comparing said base composition to one or more base compositions of amplification products obtained by performing steps a)-c) on a plurality of known organisms with known geographic origins, wherein a match identifies said geographic origin of said unknown bioagent.
21. The method of claim 20 wherein said sequences to which said at least one pair of **oligonucleotide primers** hybridize are highly conserved.
22. The method of claim 20 wherein said amplifying step comprises **polymerase chain reaction**.

23. The method of claim 20 wherein said amplifying step comprises ligase chain reaction or strand displacement amplification.
24. The method of claim 20 wherein said bioagent is a bacterium, virus, fungi, parasite, cell or spore.
25. The method of claim 20 wherein said nucleic acid is ribosomal RNA.
26. The method of claim 20 wherein said nucleic acid encodes RNase P or an RNA-dependent RNA polymerase.
27. The method of claim 20 wherein said amplification product is ionized prior to base composition determination.
28. The method of claim 20 further comprising the step of isolating nucleic acid from said bioagent prior to contacting said nucleic acid with said at least one pair of **oligonucleotide primers**.
29. The method of claim 20 further comprising the step of performing steps a)-d) using a different **oligonucleotide primer** pair and comparing the results to one or more base compositions of amplification product obtained by performing steps a)-c) on a different plurality of known organisms from those in step d).
30. The method of claim 21 wherein said one or more base composition signatures are contained in a database of base composition signatures.
31. The method of claim 21 wherein said amplification product is ionized by electrospray ionization, matrix-assisted laser desorption or fast atom bombardment.
32. The method of claim 21 wherein said base composition signature is determined by mass spectrometry.
33. The method of claim 32 wherein said mass spectrometry is selected from the group consisting of Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS), ion trap, quadrupole, magnetic sector, time of flight (TOF), q-TOF and triple quadrupole.
34. The method of claim 21 further comprising performing step b) in the presence of an analog of adenine, thymidine, guanosine or cytidine having a different molecular weight than adenosine, thymidine, guanosine or cytidine.
35. The method of claim 21 wherein said **oligonucleotide primer** comprises a base analog at positions 1 and 2 of each triplet within said **primer**, wherein said base analog binds with increased affinity to its complement compared to the native base.
36. The method of claim 35 wherein said **primer** comprises a universal base at position 3 of each triplet within said **primer**.
37. The method of claim 35 wherein said base analog is selected from the group consisting of 2,6-diaminopurine, propyne T, propyne G, phenoxazines and G-clamp.
38. The method of claim 35 wherein said universal base is selected from the group consisting of inosine, guanidine uridine, 5-nitroindole, 3-nitropyrrole, dP, dK, and 1-(2-deoxy- β -D-ribofuranosyl)-imidazole-4-carboxamide.
39. The method of claim 21 wherein said animal sample is blood, a bodily fluid, or a bodily tissue.

L5 ANSWER 22 OF 112 USPATFULL on STN

2004:158512 Methods for rapid detection and identification of bioagents in blood, bodily fluids, and bodily tissues.

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US 2004121309 A1 20040624

APPLICATION: US 2002-323233 A1 20021218 (10)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Method for detecting and identifying unknown bioagents, including bacteria, viruses and the like, by a combination of nucleic acid amplification and molecular weight determination using primers which hybridize to conserved sequence regions of nucleic acids derived from a bioagent and which bracket variable sequence regions that uniquely

(BCS) which is then matched against a database of base composition signatures, by which the bioagent is identified.

What is claimed is:

1. A method of identifying an unknown bioagent in an animal sample comprising: a) contacting nucleic acid from said bioagent in said animal sample with at least one pair of **oligonucleotide primers** which hybridize to sequences of said nucleic acid, wherein said sequences flank a variable nucleic acid sequence of the bioagent; b) amplifying said variable nucleic acid sequence to produce an amplification product; c) determining the molecular mass of said amplification product; and d) comparing said molecular mass to one or more molecular masses of amplification products obtained by performing steps a)-c) on a plurality of known organisms, wherein a match identifies said unknown bioagent in said animal sample.
2. The method of claim 1 wherein said sequences to which said at least one pair of **oligonucleotide primers** hybridize are highly conserved.
3. The method of claim 1 wherein said amplifying step comprises **polymerase chain reaction**.
4. The method of claim 1 wherein said amplifying step comprises ligase chain reaction or strand displacement amplification.
5. The method of claim 1 wherein said bioagent is a bacterium, virus, parasite, fungi, cell or spore.
6. The method of claim 1 wherein said nucleic acid is ribosomal RNA.
7. The method of claim 1 wherein said nucleic acid encodes RNase P or an RNA-dependent RNA polymerase.
8. The method of claim 1 wherein said amplification product is ionized prior to molecular mass determination.
9. The method of claim 1 further comprising the step of isolating nucleic acid from said bioagent prior to contacting said nucleic acid with said at least one pair of **oligonucleotide primers**.
10. The method of claim 1 further comprising the step of performing steps a)-d) using a different **oligonucleotide primer** pair and comparing the results to one or more molecular mass amplification products obtained by performing steps a)-c) on a different plurality of known organisms from those in step d).
11. The method of claim 1 wherein said one or more molecular masses are contained in a database of molecular masses.
12. The method of claim 1 wherein said amplification product is ionized by electrospray ionization, matrix-assisted laser desorption or fast atom bombardment.
13. The method of claim 1 wherein said molecular mass is determined by mass spectrometry.
14. The method of claim 11 wherein said mass spectrometry is selected from the group consisting of Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS), ion trap, quadrupole, magnetic sector, time of flight (TOF), Q-TOF and triple quadrupole.
15. The method of claim 1 further comprising performing step b) in the presence of an analog of adenine, thymidine, guanosine or cytidine having a different molecular weight than adenosine, thymidine, guanosine or cytidine.
16. The method of claim 1 wherein said **oligonucleotide primer** comprises a base analog at positions 1 and 2 of each triplet within said **primer**, wherein said base analog binds with increased affinity to its complement compared to the native base.
17. The method of claim 16 wherein said **primer** comprises a universal base at position 3 of each triplet within said **primer**.
18. The method of claim 16 wherein said base analog is selected from the group consisting of 2,6-diaminopurine, propyne T, propyne G, phenoxazines and G-clamp.
19. The method of claim 16 wherein said universal base is selected from the group consisting of inosine, guanidine uridine, 5-nitroindole, 3-nitropyrrole, dP, dK, and 1-(2-deoxy- β -D-ribofuranosyl)-imidazole-4-carboxamide.

fluid, or a bodily tissue.

21. A method of identifying an unknown bioagent in an animal sample comprising: a) contacting nucleic acid from said bioagent in an animal sample with at least one pair of **oligonucleotide primers** which hybridize to sequences of said nucleic acid, wherein said sequences flank a variable nucleic acid sequence; b) amplifying said variable nucleic acid sequence to produce an amplification product; c) determining the base composition of said amplification product; and d) comparing said base composition to one or more base compositions of amplification products obtained by performing steps a)-c) on a plurality of known organisms, wherein a match identifies said unknown bioagent in said animal sample.

22. The method of claim 21 wherein said sequences to which said at least one pair of **oligonucleotide primers** hybridize are highly conserved.

23. The method of claim 21 wherein said amplifying step comprises **polymerase chain reaction**.

24. The method of claim 21 wherein said amplifying step comprises ligase chain reaction or strand displacement amplification.

25. The method of claim 21 wherein said bioagent is a bacterium, virus, fungi, parasite, cell or spore.

26. The method of claim 21 wherein said nucleic acid is ribosomal RNA.

27. The method of claim 21 wherein said nucleic acid encodes RNase P or an RNA-dependent RNA polymerase.

28. The method of claim 21 wherein said amplification product is ionized prior to base composition determination.

29. The method of claim 21 further comprising the step of isolating nucleic acid from said bioagent prior to contacting said nucleic acid with said at least one pair of **oligonucleotide primers**.

30. The method of claim 21 further comprising the step of performing steps a)-d) using a different **oligonucleotide primer** pair and comparing the results to one or more base compositions of amplification product obtained by performing steps a)-c) on a different plurality of known organisms from those in step d).

31. The method of claim 21 wherein said one or more base composition signatures are contained in a database of base composition signatures.

32. The method of claim 21 wherein said amplification product is ionized by electrospray ionization, matrix-assisted laser desorption or fast atom bombardment.

33. The method of claim 21 wherein said base composition signature is determined by mass spectrometry.

34. The method of claim 33 wherein said mass spectrometry is selected from the group consisting of Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS), ion trap, quadrupole, magnetic sector, time of flight (TOF), q-TOF and triple quadrupole.

35. The method of claim 21 further comprising performing step b) in the presence of an analog of adenine, thymidine, guanosine or cytidine having a different molecular weight than adenosine, thymidine, guanosine or cytidine.

36. The method of claim 21 wherein said **oligonucleotide primer** comprises a base analog at positions 1 and 2 of each triplet within said **primer**, wherein said base analog binds with increased affinity to its complement compared to the native base.

37. The method of claim 36 wherein said **primer** comprises a universal base at position 3 of each triplet within said **primer**.

38. The method of claim 36 wherein said base analog is selected from the group consisting of 2,6-diaminopurine, propyne T, propyne G, phenoxazines and G-clamp.

39. The method of claim 36 wherein said universal base is selected from the group consisting of inosine, guanidine uridine, 5-nitroindole, 3-nitropyrrole, dP, dK, and 1-(2-deoxy- β -D-ribofuranosyl)-imidazole-4-carboxamide.

40. The method of claim 21 wherein said animal sample is blood, a bodily

41. A method of determining the absence of a bioagent in an animal sample comprising: a) contacting said animal sample suspected of containing nucleic acid encoding said bioagent with at least one pair of **oligonucleotide primers** which are capable of hybridizing to sequences of said nucleic acid, wherein said sequences flank a variable nucleic acid sequence of said bioagent; b) treating said variable nucleic acid sequence under amplification conditions capable of producing an amplification product of said variable nucleic acid sequence; c) performing spectroscopy to determine the molecular mass or base composition of all amplification products; and d) comparing said molecular mass to one or more molecular masses of amplification products or said base composition to one or more base compositions of amplification products obtained by performing steps a)-c) on a plurality of known organisms, wherein the lack of a match indicates that said bioagent is absent from said animal sample.

42. The method of claim 41 further comprising determining the presence of a positive control.

43. The method of claim 42 wherein said positive control is a known bioagent or residual **primer** signal.

L5 ANSWER 23 OF 112 USPTAFULL on STN

2004:152137 Pharmaceutical compositions and treatment methods.

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US 2004116359 A1 20040617

APPLICATION: US 2002-329065 A1 20021221 (10)

PRIORITY: US 2000-190140P 20000316 (60)

US 1999-164048P 19991108 (60)

US 1999-140028P 19990616 (60)

US 1999-126056P 19991019 (60)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention provides compositions comprising formula 1 steroids, e.g., 16 α -bromo-3 β -hydroxy-5 α -androstan-17-one hemihydrate and one or more excipients, typically wherein the composition comprises less than about 3% water. The compositions are useful to make improved pharmaceutical formulations. The invention also provides methods of intermittent dosing of steroid compounds such as analogs of 16 α -bromo-3 β -hydroxy-5 α -androstan-17-one and compositions useful in such dosing regimens. The invention further provides compositions and methods to inhibit pathogen (viral) replication, ameliorate symptoms associated with immune dysregulation and to modulate immune responses in a subject using certain steroids and steroid analogs. The invention also provides methods to make and use these immunomodulatory compositions and formulations.

CLM What is claimed is:

1. A method to treat a subject having, or susceptible to developing, a pathogen infection, an autoimmune disease, inflammation or allergy, osteoporosis, acute myelitis, sarcoidosis, a cancer, a precancer, a neurological disorder, a wound, a bone fracture, a hemorrhage, a burn, a skin lesion or an immunosuppression condition or an unwanted immune response either or both of which are associated with a chemotherapy, radiation exposure or aging, wherein the method comprises intermittent administration of an effective amount of a compound to the subject, wherein the compound is 16 α -bromo-3 β -hydroxy-5 α -androstan-17-one hemihydrate or the compound has the structure ##STR115## wherein, the dotted lines are optional double bonds and the hydrogen atom at the 5-position, if present, is in the α -configuration; R¹, R², R³, R⁴, R⁵, R⁶ and R¹⁰ independently are --H, --OH, --OR^{PR}, --SH, --SR^{PR}, .dbd.S, .dbd.CH₂, --N₃, --NH₂, --N(R^{PR})₂, --O--Si--(R¹³)₃, --CN, --NO₂, .dbd.NOH, .dbd.NOC(O)CH₃, --C(O)--CH₃, --F, --Cl, --Br, --I, an ester, a thioester, a phosphoester, a phosphothioester, a phosphonoester, a phosphiniester, a sulfite ester, a sulfate ester, an amide, an amino acid, a peptide, an ether, a thioether, an acyl group, a thioacyl group, a carbonate, a carbamate, a thioacetal, an optionally substituted alkyl group, an optionally substituted alkenyl group, an optionally substituted alkynyl group, an optionally substituted aryl moiety, an optionally substituted heteroaryl moiety, an optionally substituted monosaccharide, an optionally substituted oligosaccharide, a

more of R², R³, R⁴, R⁵, R⁶, R¹⁰, R¹⁵,
R¹⁷ and R¹⁸ independently are .dbd.O, or, R³ and both
R⁴ together comprise a structure of formula 2 ##STR16## R⁷
is --CHR10--, --CHR10--CHR10--, --CHR10--CHR10--
CHR10--, --CHR10--O--CHR10--, --CHR10--S--CHR10--
-, --CHR10--NRPR--CHR10--, --O--, --O--CHR10--,
--S--, --S--CHR10--, --NRPR-- or --NRPR--CHR10--;
R⁸ and R⁹ independently are --CHR10--,
--CHR10--CHR10--, --O--, --O--CHR10--, --S--,
--S--CHR10--, --NRPR-- or --NRPR--CHR10--, or
R⁸ or R⁹ independently is absent, leaving a 5-membered ring;
R¹³ independently are C₁₋₆ alkyl; R^{PR} independently are
a protecting group; D is a heterocycle or a 4-, 5-, 6- or 7-membered
ring that comprises saturated carbon atoms, wherein 1, 2 or 3 ring
carbon atoms of the 4-, 5-, 6- or 7-membered ring are optionally
independently substituted with --O--, --S-- or --NRPR-- or where 1,
2 or 3 hydrogen atoms of the heterocycle or 1 or 2 hydrogen atoms of the
4-, 5-, 6- or 7-membered ring are substituted with --OR^{PR},
--SR^{PR}, --N(R^{PR})₂, --O--Si--(R¹³)₃, --CN,
--NO₂, an ester, a thioester, a phosphoester, a phosphothioester, a
phosphonoester, a phosphiniester, a sulfite ester, a sulfate ester, an
amide, an amino acid, a peptide, an ether, a thioether, an acyl group, a
thioacyl group, a carbonate, a carbamate, a thioacetal, a halogen, an
optionally substituted alkyl group, an optionally substituted alkenyl
group, an optionally substituted alkynyl group, an optionally
substituted aryl moiety, an optionally substituted heteroaryl moiety, an
optionally substituted monosaccharide, an optionally substituted
oligosaccharide, a nucleoside, a nucleotide, an **oligonucleotide** or a
polymer, or, one more of the ring carbons are substituted with .dbd.O or
.dbd.S, or D comprises two 5- or 6-membered rings, wherein the rings are
fused or are linked by 1 or 2 bonds, provided that the compound is not
3 β -hydroxyandrost-5-ene-17-one, 3 β -hydroxyandrost-5-ene-1
7-one 3-sulfate or an ester or ether derivative of either compound and
provided that when the compound has the structure ##STR17## wherein
each R^A independently is --OH, .dbd.O, an ester or an ether, and
R^B is --C(O)CH₃, --OH, .dbd.O, an ester or an ether, then the
use of the medicament is for the treatment of a subject having or
susceptible to developing an autoimmune disease, inflammation or
allergy, osteoporosis, acute myelitis, sarcoidosis, a cancer, a
precancer, or an immunosuppression condition or an unwanted immune
response either or both of which are associated with a chemotherapy,
radiation exposure, a wound, a bone fracture, a hemorrhage, a skin
lesion or a burn or the medicament is for the treatment of a human
having or susceptible to developing a pathogen infection selected from
the group consisting of HIV-1, HIV-2, HTLV-1, HTLV-2, HSV-1, HSV-2,
HHV-6, HHV-8, CMV, hepatitis C virus, hepatitis B virus, Western Equine
Encephalitis Virus, Japanese Encephalitis Virus, Yellow Fever Virus, a
poxvirus, a **Dengue virus**, a papillomavirus, a togavirus, a
flavivirus, an intracellular bacterium, Mycobacterium, Listeria,
Brucella, Bartonella, Bordetella, Pseudomonas, Yersinia, Vibrio,
Salmonella, Streptococcus, Staphylococcus, Candida, Aspergillus,
Cryptococcus, Plasmodium, Trypanosoma, Leishmania, a gastrointestinal
nematode, a helminth, Cryptosporidium, Toxoplasma, Pneumocystis,
Schistosoma, or Strongyloides stercoralis.

2. The method of claim 1 wherein the compound has the structure
##STR18## wherein, hydrogen atoms at the 5 (if present), 8, 9 and 14
positions respectively in the $\alpha, \alpha, \alpha, \alpha$,
 $\alpha, \alpha, \alpha, \beta$, $\alpha, \alpha, \beta, \alpha$,
 $\alpha, \beta, \alpha, \alpha$, $\alpha, \alpha, \beta, \beta$,
 $\alpha, \beta, \alpha, \beta$, $\alpha, \beta, \beta, \alpha$ or
 $\alpha, \beta, \beta, \beta$ configurations.

3. The method of claim 2 wherein hydrogen atoms at the 5 (if present),
8, 9 and 14 positions respectively are in the
 $\alpha, \beta, \alpha, \alpha$ configurations.

4. The method of claim 1 wherein (1) R³ is a halogen and R¹,
R², and one or both R⁴ independently are --OH, --OR^{PR}, an
ether an ester having the structure steroid-O--C(O)--organic moiety,
carbonate, carbamate having the structure steroid-O--C(O)--NR^{PR}--
organic moiety, or an amino acid ester or peptide having the structure
(A) R³²--NH--{[C(R²⁹)(R³⁰)]_b--C(O)--
N(R³¹)_f--[C(R²⁹)(R³⁰)]_a--C(O)--O--steroid, (B)
R³³--O--[C(O)--[C(R²⁹)(R³⁰)]_d--N(R³¹)_g--
C(O)--[C(R²⁹)(R³⁰)]_c--N(R³¹)--O--steroid, or (C)
R³³--O--[C(O)--[C(R²⁹)(R³⁰)]_d--N(R³¹)_e--
C(O)--[C(R²⁹)(R³⁰)]_c--N(R³¹)--C(O)--O--steroid, where
each R²⁹, R³⁰ and R³¹ is independently selected and each
R²⁹ independently is --H or a C1-20 organic moiety, each R³⁰

or a protecting group, R³² and R³³ independently are --H, a protecting group, an ester or an amide where each atom or group is independently chosen, a, b, c and d independently are 1, 2, 3, 4 or 5, and e, f and g independently are an integer from 0 to 1000, or (2) R¹, R², R³ and one or both R⁴ independently are --OH, --OR^{PR}, an ether, an ester having the structure steroid-O--C(O)-organic moiety, carbonate, carbamate having the structure steroid-O--C(O)--NR^{PR}-organic moiety or an amino acid or peptide having the structure (A) R³²--NH--
{[C(R²⁹)(R³⁰)]_b--C(O)--N(R³¹)]_f--
[C(R²⁹)(R³⁰)]_a--C(O)--O-steroid, (B) R³³--O--C(O)--
[C(R²⁹)(R³⁰)]_d--N(R³¹)]_g--C(O)--
[C(R²⁹)(R³⁰)]_c--N(R³¹)]_e--O-steroid, or (C)
R³³--O--C(O)--[C(R²⁹)(R³⁰)]_d--N(R³¹)]_e--
C(O)--[C(R²⁹)(R³⁰)]_c--N(R³¹)]_e--C(O)--O-steroid, or
(3) R¹ is -H and R², R³ and one or both R⁴ are not --H, provided that the compound is not 7 α ,17 α -methyl-16-methylene-17 β -hydroxy-19-norandrost-4-ene, 7 α -methyl-16-methylene-17 β -hydroxy-17 α -ethynyl-19-norandrost-4-ene or 7 α -methyl-16-methylene-17-oxo-19-norandrost-4-ene or an ester or ether of any of these compounds, or (4) R¹ is --CN, .dbd.CH₂, acyl, thioacyl, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, ester having the structure steroid-C(O)--O-organic moiety, thioester having the structure steroid-C(S)--O-organic moiety or thioacetal having the structure steroid-C(O)--S-organic moiety, and R³ and one or both R⁴ are not --H, provided that R¹ is not optionally substituted phenyl and provided that if R¹ is --C(O)--OCH₃, then R⁴ is not --CH₃ or --C(O)--CH₃, or (5) R¹ is a halogen and R³ and one or both R⁴ are not --H, provided that either R³ is --OH, --OR^{PR}, an ether, an ester having the structure steroid-O--C(O)-organic moiety, carbonate (O--C(O)--O--), carbamate, a halogen, --NH₂, --N(R^{PR})₂, --NO₂, --N₃, .dbd.NOH, .dbd.NOC(O)CH₃, an amide, --SH, --SR^{PR}, .dbd.S, thioether, thioacetal --CN, acyl, thioacyl, or an amino acid or peptide having the structure (A) R³²--NH--
{[C(R²⁹)(R³⁰)]_b--
C(O)--N(R³¹)]_f--[C(R²⁹)(R³⁰)]_a--C(O)--O-
steroid, (B) R³³--O--C(O)--[C(R²⁹)(R³⁰)]_d--
N(R³¹)]_g--C(O)--[C(R²⁹)(R³⁰)]_c--N(R³¹)]_e--O-
steroid, or (C) R³³--O--C(O)--[C(R²⁹)(R³⁰)]_d--
N(R³¹)]_e--C(O)--[C(R²⁹)(R³⁰)]_c--N(R³¹)]_e--
C(O)--O-steroid, or one or both R⁴ independently are --OH, --OR^{PR}, an ether, an ester having the structure steroid-O--C(O)-organic moiety, carbonate, carbamate, a halogen, --NH₂, --N(R^{PR})₂, --NO₂, --N₃, .dbd.NOH, .dbd.NOC(O)CH₃, amide having the structure steroid-NR^{PR}--C(O)-organic moiety, --SH, --SR^{PR}, .dbd.S, thioether, thioacetal having the structure steroid-S--C(O)-organic moiety, --CN, alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl, ester having the structure steroid-O--C(O)-organic moiety, thioester having the structure steroid-O--C(S)-organic moiety, thioacetal having the structure steroid-S--C(O)-organic moiety, or an amino acid or peptide having the structure (A) R³²--NH--
{[C(R²⁹)(R³⁰)]_b--C(O)--N(R³¹)]_f--
[C(R²⁹)(R³⁰)]_a--C(O)--O-steroid, (B) R³³--O--C(O)--
[C(R²⁹)(R³⁰)]_d--N(R³¹)]_g--C(O)--
[C(R²⁹)(R³⁰)]_c--N(R³¹)]_e--O-steroid, or (C)
R³³--O--C(O)--[C(R²⁹)(R³⁰)]_d--N(R³¹)]_e--
C(O)--[C(R²⁹)(R³⁰)]_c--N(R³¹)]_e--C(O)--O-steroid, or
(6) R¹ is a halogen, --NH₂, --N(R^{PR})₂, --NO₂, .dbd.NOH, .dbd.NOC(O)CH₃, amide having the structure steroid-NR^{PR}--C(O)-organic moiety, carbamate having the structure steroid-NR^{PR}--C(O)--O-organic moiety, --SH, --SR^{PR}, .dbd.S, thioether, thioacetal having the structure steroid-S--C(O)-organic moiety, --CN, .dbd.CH₂, acyl, thioacyl, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl, ester having the structure steroid-C(O)--O-organic moiety, thioester having the structure steroid-C(S)--O-organic moiety or thioacetal having the structure steroid-C(O)--S-organic moiety and R², R³ and one or both R⁴ are not --H, or (7) R¹ is a halogen, --NH₂, --NO₂, --N₃, .dbd.NOH, .dbd.NOC(O)CH₃, amide having the structure steroid-NR^{PR}--C(O)-organic moiety, carbamate having the structure steroid-NR^{PR}--C(O)--O-organic moiety, --SR^{PR}, thioether, thioacetal having the structure steroid-S--C(O)-organic moiety, --CN, .dbd.CH₂, acyl, thioacyl, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl, ester having the structure steroid-C(O)--O-organic moiety, thioester having the structure steroid-C(S)--O-organic moiety, or thioacetal having the structure

not --H and R⁹ is not --CH₂--, provided that if one R⁴ is --CH₂CH₃, then R³ is not .dbd.O, or (8) R¹ is a halogen, --NH₂, --N(R^{PR})₂, --NO₂, --N₃, .dbd.NOH, .dbd.NOC(O)CH₃, amide having the structure steroid-NR^{PR}--C(O)-organic moiety, carbamate having the structure steroid-NR^{PR}--C(O)--O-organic moiety, --SR^{PR}, thioether, thioacetal having the structure steroid-S--C(O)-organic moiety, --CN, .dbd.CH₂, acyl, thioacyl, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl, ester having the structure steroid-C(O)--O-organic moiety, thioester having the structure steroid-C(S)--O-organic moiety, or thioacetal having the structure steroid-C(O)--S-organic moiety and R² and one or both R⁴ are not --H and R⁷ is not --CH₂--, or (9) R¹ is a halogen, --NH₂, --N(R^{PR})₂, --NO₂, --N₃, .dbd.NOH, .dbd.NOC(O)CH₃, amide having the structure steroid-NR^{PR}--C(O)-organic moiety, carbamate having the structure steroid-NR^{PR}--C(O)--O-organic moiety, --SR^{PR}, thioether, thioacetal having the structure steroid-S--C(O)-organic moiety, --CN, .dbd.CH₂, acyl, thioacyl, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl, ester having the structure steroid-C(O)--O-organic moiety, thioester having the structure steroid-C(S)--O-organic moiety, or thioacetal having the structure steroid-C(O)--S-organic moiety and R² and one or both R⁴ are not --H, and R⁶ is not --CH₃, provided that R¹ is not fluorine if R² is .dbd.O, one R⁴ is --OH or --O--C(O)--CH₃ and R⁶ is --CH₂OH or --CH₂--C(O)--CH₃, or (10) R¹ is --H, R² and one or both R⁴ are not --H and R⁹ is not --CH₂--, provided that R⁹ is not --C(O)-- or --CH(OH)-- when R² is --OH in the α-configuration, both R⁴ are --H and alkyl and a double bond is present at the 4-5 position, or (11) R¹ is --H, R² is not --H and R⁸ and R⁹ are not --CH₂--, or (12) R¹ is a halogen, --NH₂, --N(R^{PR})₂, --NO₂, --N₃, .dbd.NOH, .dbd.NOC(O)CH₃, amide having the structure steroid-NR^{PR}--C(O)-organic moiety, carbamate having the structure steroid-NR^{PR}--C(O)--O-organic moiety, --SH, --SR^{PR}, .dbd.S, thioether, thioacetal having the structure steroid-S--C(O)-organic moiety, --CN, .dbd.CH₂, acyl, thioacyl, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl, ester having the structure steroid-C(O)--O-organic moiety, thioester having the structure steroid-C(S)--O-organic moiety, or thioacetal having the structure steroid-C(O)--S-organic moiety and R³ and one or both R⁴ are not --H, and R⁶ is not --CH₃, or (13) the compound has the structure ##STR119## wherein R¹ is --OH, --OR^{PR}, --SH, --SR^{PR}, --N₃, --NH₂, --N(R^{PR})₂, --O--Si--(R¹³)₃, --CN, --NO₂, --C(O)--CH₃, --F, --Cl, --Br, --I, an ester, a thioester, a phosphoester, a phosphothioester, a phosphonoester, a phosphiniester, a sulfite ester, a sulfate ester, an amide, an amino acid, a peptide, an ether, a thioether, an acyl group, a thioacyl group, a carbonate, a carbamate, a thioacetal, an optionally substituted alkyl group, an optionally substituted alkenyl group, an optionally substituted alkynyl group, an optionally substituted aryl moiety, an optionally substituted heteroaryl moiety, an optionally substituted monosaccharide, an optionally substituted oligosaccharide, a nucleoside, a nucleotide, an **oligonucleotide** or a polymer, R² is --H, --OH, --OR^{PR}, --SH, --SR^{PR}, .dbd.S, --CH₂, --N₃, --NH₂, --N(R^{PR})₂, --O--Si--(R¹³)₃, --CN, --NO₂, .dbd.NOH, .dbd.NOC(O)CH₃, --C(O)--CH₃, --F, --Cl, --Br, --I, an ester, a thioester, a phosphoester, a phosphothioester, a phosphonoester, a phosphiniester, a sulfite ester, a sulfate ester, an amide, an amino acid, a peptide, an ether, a thioether, an acyl group, a thioacyl group, a carbonate, a carbamate, a thioacetal, an optionally substituted alkyl group, an optionally substituted alkenyl group, an optionally substituted alkynyl group, an optionally substituted aryl moiety, an optionally substituted heteroaryl moiety, an optionally substituted monosaccharide, an optionally substituted oligosaccharide, a nucleoside, a nucleotide, an **oligonucleotide** or a polymer, R³ is --OH, --OR^{PR}, --SH, --SR^{PR}, .dbd.S, .dbd.CH₂, --N₃, --NH₂, --N(R^{PR})₂, --O--Si--(R¹³)₃, --CN, --NO₂, .dbd.NOH, .dbd.NOC(O)CH₃, --C(O)--CH₃, --F, --Cl, --Br, --I, an ester, a thioester, a phosphoester, a phosphothioester, a phosphonoester, a phosphiniester, a sulfite ester, a sulfate ester, an amide, an amino acid, a peptide, an ether, a thioether, an acyl group, a thioacyl group, a carbonate, a carbamate, a thioacetal, an optionally substituted alkyl group, an optionally substituted alkenyl group, an optionally substituted alkynyl group, an optionally substituted aryl moiety, an optionally substituted heteroaryl moiety, an optionally substituted monosaccharide, an optionally substituted oligosaccharide, a

that the compound is not 3 α -bromo-16 α -methoxyandrost-5-ene-17-one, and R⁴ is --H, --OH, --OR^{PR}, --SH, --SR^{PR},
 .dbd.S, .dbd.CH₂, --N₃, --NH₂, --N(R^{PR})₂,
 --O--Si--(R¹³)₃, --CN, --NO₂, .dbd.NOH,
 .dbd.NOC(O)CH₃, --C(O)--CH₃, --F, --Cl, --Br, --I, an ester, a thioester, a phosphoester, a phosphothioester, a phosphonoester, a phosphiniester, a sulfite ester, a sulfate ester, an amide, an amino acid, a peptide, an ether, a thioether, an acyl group, a thioacyl group, a carbonate, a carbamate, a thioacetal, an optionally substituted alkyl group, an optionally substituted alkenyl group, an optionally substituted alkynyl group, an optionally substituted aryl moiety, an optionally substituted heteroaryl moiety, an optionally substituted monosaccharide, an optionally substituted oligosaccharide, a nucleoside, a nucleotide, an **oligonucleotide** or a polymer, or (14) the compound has the structure ##STR120## wherein R¹ is --OH, --OR^{PR}, --SH, --SR^{PR}, --N₃, --NH₂, --N(R^{PR})₂, --O--Si--(R¹³)₃, --CN, --NO₂, --C(O)--CH₃, --F, --Cl, --Br, --I, an ester, a thioester, a phosphoester, a phosphothioester, a phosphonoester, a phosphiniester, a sulfite ester, a sulfate ester, an amide, an amino acid, a peptide, an ether, a thioether, an acyl group, a thioacyl group, a carbonate, a carbamate, a thioacetal, an optionally substituted alkyl group, an optionally substituted alkenyl group, an optionally substituted alkynyl group, an optionally substituted aryl moiety, an optionally substituted heteroaryl moiety, an optionally substituted monosaccharide, an optionally substituted oligosaccharide, a nucleoside, a nucleotide, an **oligonucleotide** or a polymer, R² is --OH, --OR^{PR}, --SH, --SR^{PR}, .dbd.S, .dbd.CH₂, --N₃, --NH₂, --N(R^{PR})₂, --O--Si--(R¹³)₃, --CN, --NO₂, .dbd.NOH, .dbd.NOC(O)CH₃, --C(O)--CH₃, --F, --Cl, --Br, --I, an ester, a thioester, a phosphoester, a phosphothioester, a phosphonoester, a phosphiniester, a sulfite ester, a sulfate ester, an amide, an amino acid, a peptide, an ether, a thioether, an acyl group, a thioacyl group, a carbonate, a carbamate, a thioacetal, an optionally substituted alkyl group, an optionally substituted alkenyl group, an optionally substituted alkynyl group, an optionally substituted aryl moiety, an optionally substituted heteroaryl moiety, an optionally substituted monosaccharide, an optionally substituted oligosaccharide, a nucleoside, a nucleotide, an **oligonucleotide** or a polymer, and R³ is --H, --OH, --OR^{PR}, --SH, --SR^{PR}, .dbd.S, .dbd.CH₂, --N₃, --NH₂, --N(R^{PR})₂, --O--Si--(R¹³)₃, --CN, --NO₂, .dbd.NOH, .dbd.NOC(O)CH₃, --C(O)--CH₃, --F, --Cl, --Br, --I, an ester, a thioester, a phosphoester, a phosphothioester, a phosphonoester, a phosphiniester, a sulfite ester, a sulfate ester, an amide, an amino acid, a peptide, an ether, a thioether, an acyl group, a thioacyl group, a carbonate, a carbamate, a thioacetal, an optionally substituted alkyl group, an optionally substituted alkenyl group, an optionally substituted alkynyl group, an optionally substituted aryl moiety, an optionally substituted heteroaryl moiety, an optionally substituted monosaccharide, an optionally substituted oligosaccharide, a nucleoside, a nucleotide, an **oligonucleotide** or a polymer, provided that the compound is not 3 α -bromo-16 α -methoxyandrost-5-ene-17-one, and R⁴ is --H, --OH, --OR^{PR}, --SH, --SR^{PR}, .dbd.S, .dbd.CH₂, --N₃, --NH₂, --N(R^{PR})₂, --O--Si--(R¹³)₃, --CN, --NO₂, .dbd.NOH, .dbd.NOC(O)CH₃, --C(O)--CH₃, --F, --Cl, --Br, --I, an ester, a thioester, a phosphoester, a phosphothioester, a phosphonoester, a phosphiniester, a sulfite ester, a sulfate ester, an amide, an amino acid, a peptide, an ether, a thioether, an acyl group, a thioacyl group, a carbonate, a carbamate, a thioacetal, an optionally substituted alkyl group, an optionally substituted alkenyl group, an optionally substituted alkynyl group, an optionally substituted aryl moiety, an optionally substituted heteroaryl moiety, an optionally substituted monosaccharide, an optionally substituted oligosaccharide, a nucleoside, a nucleotide, an **oligonucleotide** or a polymer, or (15) R¹ is a halogen, --NH₂, --N(R^{PR})₂, --NO₂, --N₃, .dbd.NOH, amide having the structure steroid-NR^{PR}--C(O)-organic moiety, carbamate having the structure steroid-NR^{PR}--C(O)--O-organic moiety, --SR^{PR}, thioether, thioacetal having the structure steroid-S--C(O)-organic moiety, --CN, .dbd.CH₂, acyl, thioacyl, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl, ester having the structure steroid-C(O)--O-organic moiety, thioester having the structure steroid-C(S)--O-organic moiety, or thioacetal having the structure steroid-C(O)--S-organic moiety and R², one or both R⁴ and R⁷ are not --H or --CH₂--, provided that if R¹ is --NH₂ or --N(R^{PR})₂, then R² is not methyl, or (16) R¹ is --H and R³, one or both R⁴ are not --H and R⁵ is not --CH₂--, or (17) R¹ is --H and R³, one or both

--H and R², one or both R⁴ are not --H and R⁸ is not --CH₂--, or (19) R¹ is a halogen, R² and R⁸ are not --H or --CH₂-- and one or both R⁴ independently are --OR^{PR}, ether, an ester having the structure steroid-O--C(O)-organic moiety, carbonate (O--C(O)--O--), carbamate having the structure steroid-O--C(O)--NR^{PR}-organic moiety, optionally substituted monosaccharide, optionally substituted oligosaccharide, a nucleoside, a nucleotide, an **oligonucleotide**, a polymer, or an amino acid or peptide having the structure (A) R³²--NH--{[C(R²⁹)(R³⁰)]_b--C(O)--N(R³¹)}_f--[C(R²⁹)(R³⁰)]_a--C(O)--O-steroid, (B) R³³--O--{C(O)--[C(R²⁹)(R³⁰)]_d--N(R³¹)}_g--C(O)--[C(R²⁹)(R³⁰)]_c--N(R³¹)--O-steroid, or (C) R³³--O--{C(O)--[C(R²⁹)(R³⁰)]_d--N(R³¹)}_e--C(O)--[C(R²⁹)(R³⁰)]_c--N(R³¹)--C(O)--O-steroid.

5. The method of claim 4 wherein hydrogen atoms at the 5 (if present), 8, 9 and 14 positions respectively are in the $\alpha,\beta,\alpha,\alpha$ configurations.

6. The method of claim 1 wherein the compound has the structure ##STR121## wherein, R⁵ and R⁶ independently are --CH₃, --H or --CH₂OH, R⁷, R⁸ and R⁹ independently are --CH₂--, --O--, --NH-- or --S--, R¹, R², R³ and R⁴ respectively are in the β,β,α,β , $\alpha,\beta,\alpha,\beta$, $\beta,\alpha,\alpha,\beta$, β,β,β,β or $\beta,\beta,\alpha,\alpha$ configurations and the compound's structure is designated by numbers assigned to R¹, R², R³ and R⁴ according to the convention, R¹.R².R³.R⁴, wherein the structures for R¹, R², R³ and R⁴ are designated by numbers respectively and, for R¹, structure 1 is --OH, structure 3 is --SH, structure 4 is .dbd.S, structure 5 is --OCH₃, structure 6 is --O--S(O)(O)--O--Na⁺, structure 7 is --O--S(O)(O)--OC₂H₅, structure 8 is --CH₃, structure 9 is --H, and structure 10 is --OC(O)C(CH₃)₃, and for R², structure 1 is --H, structure 2 is --OH, structure 3 is .dbd.O, structure 4 is --CH₃, structure 5 is --OCH₃, structure 6 is --OC₂H₅, structure 7 is --OCH₂CH₂CH₃, structure 8 is --OCH₂CH₂CH₂CH₃, structure 9 is --Cl, and structure 10 is --Br, and for R³, structure 1 is --Br, structure 2 is --Cl, structure 3 is --I, structure 4 is --F, structure 5 is --H, structure 6 is --OH, structure 7 is =O, structure 8 is --OC(O)CH₃, structure 9 is --OC(O)CH₂CH₃, and structure 10 is --OC(O)CH₂CH₂CH₃, and for R⁴, structure 1 is .dbd.O, structure 2 is --OH, structure 3 is --H, structure 4 is --F, structure 5 is --Cl, structure 6 is --Br, structure 7 is --I, structure 8 is --OC(O)CH₃, structure 9 is --OC(O)CH₂CH₃, and structure 10 is --OC(O)CH₂CH₂CH₃, wherein the compound is 1.1.1.1, 1.1.1.2, 1.1.1.3, 1.1.1.4, 1.1.1.5, 1.1.1.6, 1.1.1.7, 1.1.1.8, 1.1.1.9, 1.1.1.10, 1.1.2.1, 1.1.2.2, 1.1.2.3, 1.1.2.4, 1.1.2.5, 1.1.2.6, 1.1.2.7, 1.1.2.8, 1.1.2.9, 1.1.2.10, 1.1.3.1, 1.1.3.2, 1.1.3.3, 1.1.3.4, 1.1.3.5, 1.1.3.6, 1.1.3.7, 1.1.3.8, 1.1.3.9, 1.1.3.10, 1.1.4.1, 1.1.4.2, 1.1.4.3, 1.1.4.4, 1.1.4.5, 1.1.4.6, 1.1.4.7, 1.1.4.8, 1.1.4.9, 1.1.4.10, 1.1.5.1, 1.1.5.2, 1.1.5.3, 1.1.5.4, 1.1.5.5, 1.1.5.6, 1.1.5.7, 1.1.5.8, 1.1.5.9, 1.1.5.10, 1.1.6.1, 1.1.6.2, 1.1.6.3, 1.1.6.4, 1.1.6.5, 1.1.6.6, 1.1.6.7, 1.1.6.8, 1.1.6.9, 1.1.6.10, 1.1.7.1, 1.1.7.2, 1.1.7.3, 1.1.7.4, 1.1.7.5, 1.1.7.6, 1.1.7.7, 1.1.7.8, 1.1.7.9, 1.1.7.10, 1.1.8.1, 1.1.8.2, 1.1.8.3, 1.1.8.4, 1.1.8.5, 1.1.8.6, 1.1.8.7, 1.1.8.8, 1.1.8.9, 1.1.8.10, 1.1.9.1, 1.1.9.2, 1.1.9.3, 1.1.9.4, 1.1.9.5, 1.1.9.6, 1.1.9.7, 1.1.9.8, 1.1.9.9, 1.1.9.10, 1.1.10.1, 1.1.10.2, 1.1.10.3, 1.1.10.4, 1.1.10.5, 1.1.10.6, 1.1.10.7, 1.1.10.8, 1.1.10.9, 1.1.10.10, 1.2.1.1, 1.2.1.2, 1.2.1.3, 1.2.1.4, 1.2.1.5, 1.2.1.6, 1.2.1.7, 1.2.1.8, 1.2.1.9, 1.2.1.10, 1.2.2.1, 1.2.2.2, 1.2.2.3, 1.2.2.4, 1.2.2.5, 1.2.2.6, 1.2.2.7, 1.2.2.8, 1.2.2.9, 1.2.2.10, 1.2.3.1, 1.2.3.2, 1.2.3.3, 1.2.3.4, 1.2.3.5, 1.2.3.6, 1.2.3.7, 1.2.3.8, 1.2.3.9, 1.2.3.10, 1.2.4.1, 1.2.4.2, 1.2.4.3, 1.2.4.4, 1.2.4.5, 1.2.4.6, 1.2.4.7, 1.2.4.8, 1.2.4.9, 1.2.4.10, 1.2.5.1, 1.2.5.2, 1.2.5.3, 1.2.5.4, 1.2.5.5, 1.2.5.6, 1.2.5.7, 1.2.5.8, 1.2.5.9, 1.2.5.10, 1.2.6.1, 1.2.6.2, 1.2.6.3, 1.2.6.4, 1.2.6.5, 1.2.6.6, 1.2.6.7, 1.2.6.8, 1.2.6.9, 1.2.6.10, 1.2.7.1, 1.2.7.2, 1.2.7.3, 1.2.7.4, 1.2.7.5, 1.2.7.6, 1.2.7.7, 1.2.7.8, 1.2.7.9, 1.2.7.10, 1.2.8.1, 1.2.8.2, 1.2.8.3, 1.2.8.4, 1.2.8.5, 1.2.8.6, 1.2.8.7, 1.2.8.8, 1.2.8.9, 1.2.8.10, 1.2.9.1, 1.2.9.2, 1.2.9.3, 1.2.9.4, 1.2.9.5, 1.2.9.6, 1.2.9.7, 1.2.9.8, 1.2.9.9, 1.2.9.10, 1.2.10.1, 1.2.10.2, 1.2.10.3, 1.2.10.4, 1.2.10.5, 1.2.10.6, 1.2.10.7, 1.2.10.8, 1.2.10.9, 1.2.10.10, 1.3.1.1, 1.3.1.2, 1.3.1.3, 1.3.1.4, 1.3.1.5, 1.3.1.6, 1.3.1.7, 1.3.1.8, 1.3.1.9, 1.3.1.10, 1.3.2.1, 1.3.2.2, 1.3.2.3, 1.3.2.4, 1.3.2.5, 1.3.2.6, 1.3.2.7, 1.3.2.8, 1.3.2.9, 1.3.2.10, 1.3.3.1, 1.3.3.2, 1.3.3.3, 1.3.3.4, 1.3.3.5, 1.3.3.6, 1.3.3.7, 1.3.3.8, 1.3.3.9, 1.3.3.10, 1.3.4.1, 1.3.4.2, 1.3.4.3, 1.3.4.4, 1.3.4.5, 1.3.4.6, 1.3.4.7, 1.3.4.8, 1.3.4.9, 1.3.4.10, 1.3.5.1, 1.3.5.2, 1.3.5.3, 1.3.5.4,

[illegible]

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3.2.2.3, 3.2.2.4, 3.2.2.5, 3.2.2.6, 3.2.2.7, 3.2.2.8, 3.2.2.9, 3.2.2.10,
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3.3.4.8, 3.3.4.9, 3.3.4.10, 3.3.5.1, 3.3.5.2, 3.3.5.3, 3.3.5.4, 3.3.5.5,
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3.3.8.8, 3.3.8.9, 3.3.8.10, 3.3.9.1, 3.3.9.2, 3.3.9.3, 3.3.9.4, 3.3.9.5,
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3.3.10.10, 3.4.1.1, 3.4.1.2, 3.4.1.3, 3.4.1.4, 3.4.1.5, 3.4.1.6,
3.4.1.7, 3.4.1.8, 3.4.1.9, 3.4.1.10, 3.4.2.1, 3.4.2.2, 3.4.2.3, 3.4.2.4,
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9.6.1.7, 9.6.1.8, 9.6.1.9, 9.6.1.10, 9.6.2.1, 9.6.2.2, 9.6.2.3, 9.6.2.4,
9.6.2.5, 9.6.2.6, 9.6.2.7, 9.6.2.8, 9.6.2.9, 9.6.2.10, 9.6.3.1, 9.6.3.2,
9.6.3.3, 9.6.3.4, 9.6.3.5, 9.6.3.6, 9.6.3.7, 9.6.3.8, 9.6.3.9, 9.6.3.10,
9.6.4.1, 9.6.4.2, 9.6.4.3, 9.6.4.4, 9.6.4.5, 9.6.4.6, 9.6.4.7, 9.6.4.8,
9.6.4.9, 9.6.4.10, 9.6.5.1, 9.6.5.2, 9.6.5.3, 9.6.5.4, 9.6.5.5, 9.6.5.6,
9.6.5.7, 9.6.5.8, 9.6.5.9, 9.6.5.10, 9.6.6.1, 9.6.6.2, 9.6.6.3, 9.6.6.4,
9.6.6.5, 9.6.6.6, 9.6.6.7, 9.6.6.8, 9.6.6.9, 9.6.6.10, 9.6.7.1, 9.6.7.2,
9.6.7.3, 9.6.7.4, 9.6.7.5, 9.6.7.6, 9.6.7.7, 9.6.7.8, 9.6.7.9, 9.6.7.10,
9.6.8.1, 9.6.8.2, 9.6.8.3, 9.6.8.4, 9.6.8.5, 9.6.8.6, 9.6.8.7, 9.6.8.8,
9.6.8.9, 9.6.8.10, 9.6.9.1, 9.6.9.2, 9.6.9.3, 9.6.9.4, 9.6.9.5, 9.6.9.6,
9.6.9.7, 9.6.9.8, 9.6.9.9, 9.6.9.10, 9.6.10.1, 9.6.10.2, 9.6.10.3,
9.6.10.4, 9.6.10.5, 9.6.10.6, 9.6.10.7, 9.6.10.8, 9.6.10.9, 9.6.10.10,
9.7.1.1, 9.7.1.2, 9.7.1.3, 9.7.1.4, 9.7.1.5, 9.7.1.6, 9.7.1.7, 9.7.1.8,
9.7.1.9, 9.7.1.10, 9.7.2.1, 9.7.2.2, 9.7.2.3, 9.7.2.4, 9.7.2.5, 9.7.2.6,
9.7.2.7, 9.7.2.8, 9.7.2.9, 9.7.2.10, 9.7.3.1, 9.7.3.2, 9.7.3.3, 9.7.3.4,
9.7.3.5, 9.7.3.6, 9.7.3.7, 9.7.3.8, 9.7.3.9, 9.7.3.10, 9.7.4.1, 9.7.4.2,
9.7.4.3, 9.7.4.4, 9.7.4.5, 9.7.4.6, 9.7.4.7, 9.7.4.8, 9.7.4.9, 9.7.4.10
9.7.5.1, 9.7.5.2, 9.7.5.3, 9.7.5.4, 9.7.5.5, 9.7.5.6, 9.7.5.7, 9.7.5.8,
9.7.5.9, 9.7.5.10, 9.7.6.1, 9.7.6.2, 9.7.6.3, 9.7.6.4, 9.7.6.5, 9.7.6.6,
9.7.6.7, 9.7.6.8, 9.7.6.9, 9.7.6.10, 9.7.7.1, 9.7.7.2, 9.7.7.3, 9.7.7.4,
9.7.7.5, 9.7.7.6, 9.7.7.7, 9.7.7.8, 9.7.7.9, 9.7.7.10, 9.7.8.1, 9.7.8.2,
9.7.8.3, 9.7.8.4, 9.7.8.5, 9.7.8.6, 9.7.8.7, 9.7.8.8, 9.7.8.9, 9.7.8.10,
9.7.9.1, 9.7.9.2, 9.7.9.3, 9.7.9.4, 9.7.9.5, 9.7.9.6, 9.7.9.7, 9.7.9.8,
9.7.9.9, 9.7.9.10, 9.7.10.1, 9.7.10.2, 9.7.10.3, 9.7.10.4, 9.7.10.5,
9.7.10.6, 9.7.10.7, 9.7.10.8, 9.7.10.9, 9.7.10.10, 9.8.1.1, 9.8.1.2,
9.8.1.3, 9.8.1.4, 9.8.1.5, 9.8.1.6, 9.8.1.7, 9.8.1.8, 9.8.1.9, 9.8.1.10,
9.8.2.1, 9.8.2.2, 9.8.2.3, 9.8.2.4, 9.8.2.5, 9.8.2.6, 9.8.2.7, 9.8.2.8,
9.8.2.9, 9.8.2.10, 9.8.3.1, 9.8.3.2, 9.8.3.3, 9.8.3.4, 9.8.3.5, 9.8.3.6,
9.8.3.7, 9.8.3.8, 9.8.3.9, 9.8.3.10, 9.8.4.1, 9.8.4.2, 9.8.4.3, 9.8.4.4,
9.8.4.5, 9.8.4.6, 9.8.4.7, 9.8.4.8, 9.8.4.9, 9.8.4.10, 9.8.5.1, 9.8.5.2,
9.8.5.3, 9.8.5.4, 9.8.5.5, 9.8.5.6, 9.8.5.7, 9.8.5.8, 9.8.5.9, 9.8.5.10,
9.8.6.1, 9.8.6.2, 9.8.6.3, 9.8.6.4, 9.8.6.5, 9.8.6.6, 9.8.6.7, 9.8.6.8,
9.8.6.9, 9.8.6.10, 9.8.7.1, 9.8.7.2, 9.8.7.3, 9.8.7.4, 9.8.7.5, 9.8.7.6,
9.8.7.7, 9.8.7.8, 9.8.7.9, 9.8.7.10, 9.8.8.1, 9.8.8.2, 9.8.8.3, 9.8.8.4,
9.8.8.5, 9.8.8.6, 9.8.8.7, 9.8.8.8, 9.8.8.9, 9.8.8.10, 9.8.9.1, 9.8.9.2,
9.8.9.3, 9.8.9.4, 9.8.9.5, 9.8.9.6, 9.8.9.7, 9.8.9.8, 9.8.9.9, 9.8.9.10,
9.8.10.1, 9.8.10.2, 9.8.10.3, 9.8.10.4, 9.8.10.5, 9.8.10.6, 9.8.10.7,
9.8.10.8, 9.8.10.9, 9.8.10.10, 9.9.1.1, 9.9.1.2, 9.9.1.3, 9.9.1.4,
9.9.1.5, 9.9.1.6, 9.9.1.7, 9.9.1.8, 9.9.1.9, 9.9.1.10, 9.9.2.1, 9.9.2.2,
9.9.2.3, 9.9.2.4, 9.9.2.5, 9.9.2.6, 9.9.2.7, 9.9.2.8, 9.9.2.9, 9.9.2.10,
9.9.3.1, 9.9.3.2, 9.9.3.3, 9.9.3.4, 9.9.3.5, 9.9.3.6, 9.9.3.7, 9.9.3.8,
9.9.3.9, 9.9.3.10, 9.9.4.1, 9.9.4.2, 9.9.4.3, 9.9.4.4, 9.9.4.5, 9.9.4.6,
9.9.4.7, 9.9

[illegible]

respectively are in the β,β,β,β configurations.

8. The method of claim 6 wherein R¹, R², R³ and R⁴ respectively are in the β,β,β,β configurations.

9. The method of claim 6 wherein R¹, R², R³ and R⁴ respectively are in the $\alpha,\beta,\alpha,\beta$ configurations.

10. The method of claim 6 wherein no double bond is present at the 1-2 or 5-6 positions, R¹, R², R³ and R⁴ respectively are in the β,β,α,β configurations, R⁵ and R⁶ are --CH₃ and R⁷, R⁸ and R⁹ are --CH₂--.

11. The method of claim 6 wherein no double bond is present at the 1-2 position, a double bond is present at the 5-6 position, R¹, R², R³ and R⁴ respectively are in the β,β,α,β configurations, R⁵ and R⁶ are --CH₃ and R⁷, R⁸ and R⁹ are --CH₂--.

12. The method of claim 6 wherein no double bond is present at the 1-2 position, a double bond is present at the 5-6 position, R¹, R², R³ and R⁴ respectively are in the $\beta,\alpha,\alpha,\beta$ configurations, R⁵ and R⁶ are --CH₃ and R⁷, R⁸ and R⁹ are --CH₂--.

13. The method of claim 6 wherein no double bond is present at the 5-6 position, a double bond is present at the 1-2 position, R¹, R², R³ and R⁴ respectively are in the $\alpha,\beta,\alpha,\beta$ configurations, R⁵ and R⁶ are --CH₃ and R⁷, R⁸ and R⁹ are --CH₂--.

12. The method of claim 6 wherein R⁸ is --O-- or --NH-- and R⁷ and R⁹ are --CH₂--.

13. The method of claim 6 wherein R⁹ is --O-- or --NH-- and R⁷ and R⁸ are --CH₂--.

14. The method of claim 6 wherein R⁷, R⁸ and R⁹ are --CH₂-- and no double bond is present at the 1-2 or 5-6 positions.

15. The method of claim 6 wherein R⁷, R⁸ and R⁹ are --CH₂--, no double bond is present at the 1-2 position and a double bond is present at the 5-6 position.

16. The method of claim 6 wherein R⁷, R⁸ and R⁹ are --CH₂--, a double bond is present at the 1-2 position and no double bond is present at the 5-6 position.

17. The method of claim 6 wherein the compound is 16 β -bromo-3 β -hydroxy-5 α -androstan-17-one, 16 α -bromo-3 β -hydroxy-5 α -androstan-17-one, 3 β ,16 β -dihydroxy-5 α -androstan-17-one, 3 β ,16 β -dihydroxy-5 α -androstan-17-one, 3 β ,16 α ,17 β -trihydroxy-5 α -androstan-17-one, 3 β ,16 β ,17 β -trihydroxy-5 α -androstan-17-one or 3 α ,16 α ,17 β -trihydroxy-5 α -androstan-17-one.

18. The method of claim 6 wherein the compound is 3 β -hydroxy-16 α -fluoro-5 α -androstan-17-one, 16 α -fluoroandrosterone-17-one, 7 β -hydroxy-16 α -fluoroandrosterone-5-ene-17-one, 7 α -hydroxy-16 α -fluoroandrosterone-5-ene-17-one, 3 α -hydroxy-16 α -fluoroandrosterone-5-ene-17-one, 3 α ,17 β -dihydroxy-16 α -fluoroandrosterone-5-ene, 3 α ,7 β ,17 β -trihydroxy-16 α -fluoroandrosterone-5-ene, 3 α ,17 β -dihydroxy-7-oxo-16 α -fluoroandrosterone-5-ene, 7,17-dioxo-16 α -fluoroandrosterone-5-ene or 17 β -hydroxy-7-oxo-16 α -fluoroandrosterone-5-ene.

19. The method of claim 6 wherein the compound is 3 β ,17 β -dihydroxyandrosterone-5-ene, 3 β ,7 β ,17 β -trihydroxyandrosterone-5-ene, 3 β ,7 β ,17 β -trihydroxy-16-oxoandrosterone-5-ene, 3 β ,7 α ,17 β -trihydroxyandrosterone-5-ene, 3 β ,16 α ,17 β -trihydroxyandrosterone-5-ene, 3 β ,17 β -dihydroxy-7-oxoandrosterone-5-ene, 3 β ,7 β ,16 α ,17 β -tetrahydroxyandrosterone-5-ene or 3 β ,7 β ,17 β -trihydroxy-16 α -bromoandrosterone-5-ene.

20. The method of claim 17 wherein the compound is 16 α -bromo-

21. The method of claim 1 wherein the compound is 16 α -bromo-3 β -hydroxy-5 α -androst-17-one hemihydrate.

22. The method of claim 19 wherein the compound is 3 β ,17 β -dihydroxyandrost-5-ene.

23. The method of claim 19 wherein the compound is 3 β ,7 β ,17 β -trihydroxyandrost-5-ene.

24. The method of claim 19 wherein the compound is 3 α ,17 β -dihydroxy-16 α -fluoroandrost-5-ene.

25. The method of claim 17 wherein the compound is 16 β -bromo-3 β -hydroxy-5 α -androst-17-one.

26. The method of claim 17 wherein the compound is 3 β ,16 α -dihydroxy-5 α -androst-17-one or 3 α ,16 α ,17 β -trihydroxy-5 α -androstane.

27. The method of claim 1 wherein the intermittent dosing protocol comprises: (a) administering the compound to the subject at least once per day for at least 2 days; (b) not administering the one or more formula 1 compounds to the subject for at least 1 day; (c) administering the one or more formula 1 compounds to the subject at least once per day for at least 2 days; and (d) optionally repeating steps (a), (b) and (c) at least once or variations of steps (a), (b) and (c) at least once.

28. The method of claim 27 wherein step (c) comprises the same dosing period as step (a).

29. The method of claim 28 wherein step (a) is administering the compound for about 3-24 days.

30. The method of claim 28 wherein step (b) is not administering the compound for about 3-120 days.

31. The method of claim 29 wherein step (b) is not administering the compound for about 3-120 days.

32. The method of claim 29 wherein step (b) comprises not administering the compound for about 4-60 days.

33. The method of claim 27 wherein step (b) comprises not administering the compound for about 4-60 days.

34. The method of claim 31 wherein the compound is 16 β -bromo-3 β -hydroxy-5 α -androst-17-one, 16 α -bromo-3 β -hydroxy-5 α -androst-17-one, 3 β ,16 α -dihydroxy-5 α -androst-17-one, 3 β ,16 β -dihydroxy-5 α -androst-17-one, 3 β ,16 α ,17 β -trihydroxy-5 α -androstane, 3 β ,16 β ,17 β -trihydroxy-5 α -androstane, 3 α ,16 α ,17 β -trihydroxy-5 α -androstane, 16 α -fluoro-3 β -hydroxy-5 α -androst-17-one, 16 α -fluoroandrost-5-ene-17-one, 7 β -hydroxy-16 α -fluoroandrost-5-ene-17-one, 7 α -hydroxy-16 α -fluoroandrost-5-ene-17-one, 3 α -hydroxy-16 α -fluoroandrost-5-ene-17-one, 3 α ,17 β -dihydroxy-16 α -fluoroandrost-5-ene, 3 α ,7 β ,17 β -trihydroxy-16 α -fluoroandrost-5-ene, 3 β ,17 β -dihydroxyandrost-5-ene, 3 β ,7 β ,17 β -trihydroxyandrost-5-ene, 3 β ,7 α ,17 β -trihydroxyandrost-5-ene, 3 β ,16 α ,17 β -trihydroxyandrost-5-ene, 3 β ,17 β -dihydroxy-7-oxoandrost-5-ene, 3 β ,7 β ,16 α ,17 β -tetrahydroxyandrost-5-ene or 3 β ,7 β ,17 β -trihydroxy-16 α -bromoandrost-5-ene.

35. The method of claim 31 wherein the compound is 16 β -bromo-3 β -hydroxy-5 α -androst-17-one, 16 α -bromo-3 β -hydroxy-5 α -androst-17-one, 3 β ,16 α -dihydroxy-5 α -androst-17-one, 3 β ,16 β -dihydroxy-5 α -androst-17-one, 3 β ,16 α ,17 β -trihydroxy-5 α -androstane, 3 β ,16 β ,17 β -trihydroxy-5 α -androstane or 16 α -fluoro-3 β -hydroxy-5 α -androst-17-one.

36. The method of claim 31 wherein the compound is 3 β ,17 β -dihydroxyandrost-5-ene, 3 β ,7 β ,17 β -trihydroxyandrost-5-ene,

3 β ,16 α ,17 β -trihydroxyandrost-5-ene, 3 β ,17 β -dihydroxy-7-oxoandrost-5-ene, 3 β ,7 β ,16 α ,17 β -tetrahydroxyandrost-5-ene or 3 β ,7 β ,17 β -trihydroxy-16 α -bromoandrost-5-ene.

37. The method of claim 35 wherein the compound is 16 α -bromo-3 β -hydroxy-5 α -androst-17-one.

38. The method of claim 37 wherein the 16 α -bromo-3 β -hydroxy-5 α -androst-17-one comprises 16 α -bromo-3 β -hydroxy-5 α -androst-17-one hemihydrate.

39. The method of claim 36 wherein the compound is 3 β ,7 β ,17 β -dihydroxyandrost-5-ene.

40. The method of claim 36 wherein the compound is 3 β ,7 β ,17 β -trihydroxyandrost-5-ene.

41. The method of claim 34 wherein the compound is 3 α ,17 β -dihydroxy-16 α -fluoroandrost-5-ene.

42. The method of claim 35 wherein the compound is 16 β -bromo-3 β -hydroxy-5 α -androst-17-one.

43. The method of claim 35 wherein the compound is 3 β ,16 α -dihydroxy-5 α -androst-17-one.

44. The method of claim 34, wherein the subject has a pathogen infection.

45. The method of claim 44, wherein the pathogen infection is a viral infection.

46. The method of claim 45, wherein the viral infection is a retrovirus infection, optionally selected from the group consisting of a HIV-1 infection and a HIV-2 infection.

47. The method of claim 45 where the infection is a hepatitis B virus infection, a hepatitis C virus infection, a poxvirus infection or a papillomavirus infection.

48. The method of claim 44, wherein the pathogen infection is a parasite infection.

49. The method of claim 48, wherein the parasite infection is a Plasmodium infection, a Trypanosoma infection, a Leishmania infection, a Schistosoma infection or a Cryptosporidium infection.

50. The method of claim 44, wherein the pathogen infection is a bacterial, fungal or yeast infection.

51. The method of claim 50, wherein the bacterial, fungal or yeast infection is an intracellular bacterium infection, a Mycobacterium infection, a Brucella infection, a Pseudomonas infection, a Yersinia infection, a Vibrio infection, a Salmonella infection, a Candida infection, an Aspergillus infection or a Cryptococcus infection.

52. The method of claim 34 wherein the subject has a cancer or a precancer.

53. The method of claim 52 wherein the cancer or precancer is a cancer or precancer arising in the throat, esophagus, stomach, intestine, colon, lung, or central nervous system.

54. The method of claim 34, wherein the subject has, or is subject to developing, an immunosuppression condition or an unwanted immune response either or both of which are associated with a chemotherapy, or radiation exposure.

55. The method of claim 54, wherein the chemotherapy or radiation exposure is a cyclosporin, cyclohexamide, mitomycin C, adriamycin, taxol, amphotericin B, cis-platin, a protease inhibitor, a nucleoside analog, a nucleotide analog or a corticosteroid treatment or γ -radiation therapy.

56. The method of claim 34, wherein the subject has a wound, osteoporosis, a bone fracture, a hemorrhage or a burn.

57. The method of claim 34, wherein the subject has a neurological

58. The method of claim 57, wherein the neurological disorder is AIDS associated dementia, Alzheimer's disease, Parkinson's disease, schizophrenia or multiple sclerosis.

59. A method to administer a compound to a subject, wherein the method comprises intermittent administration of about 0.05 mg/kg to about 30 mg/kg per day of a compound to the subject, wherein the compound is

16 β -bromo-3 β -hydroxy-5 α -androst-17-one,
16 α -bromo-3 β -hydroxy-5 α -androst-17-one,
3 β ,16 α -dihydroxy-5 α -androst-17-one,
3 β ,16 β -dihydroxy-5 α -androst-17-one,
3 β ,16 α ,17 β -trihydroxy-5 α -androstane,
3 β ,16 β ,17 β -trihydroxy-5 α -androstane,
16 α -fluoro-3 β -hydroxy-5 α -androst-17-one,
16 α -fluoroandrost-5-ene-17-one, 7 β -hydroxy-16 α -
fluoroandrost-5-ene-17-one, 7 α -hydroxy-16 α -fluoroandrost-5-
ene-17-one, 3 α -hydroxy-16 α -fluoroandrost-5-ene-17-one,
3 α ,17 β -dihydroxy-16 α -fluoroandrost-5-ene,
3 α ,7 β ,17 β -trihydroxy-16 α -fluoroandrost-5-ene,
3 β ,17 β -dihydroxyandrost-5-ene, 3 β ,7 β ,17 β -
trihydroxyandrost-5-ene, 3 β ,7 α ,17 β -trihydroxyandrost-5-
ene, 3 β ,16 α ,17 β -trihydroxyandrost-5-ene,
3 β ,17 β -dihydroxy-7-oxoandrost-5-ene,
3 β ,7 β ,16 β ,17 β -tetrahydroxyandrost-5-ene or
3 β ,7 β ,17 β -trihydroxy-16 α -bromoandrost-5-ene.

60. The method of claim 59 wherein the subject is a human.

61. The method of claim 59 wherein the intermittent dosing protocol comprises the steps of: (a) administering the compound(s) to the subject at least once per day for at least 2 days; (b) not administering the compound(s) to the subject for at least 1 day; (c) administering the compound(s) to the subject at least once per day for at least 2 days; and (d) optionally repeating steps (a), (b) and (c) at least once or variations of steps (a), (b) and (c) at least once, and wherein the subject is a human.

62. The method of claim 61 wherein step (c) comprises the same dosing regimen as step (a).

63. The method of claim 61 wherein step (a) is administering the compound for about 3-24 days.

64. The method of claim 62 wherein step (a) is administering the compound for about 3-24 days.

65. The method of claim 62 wherein step (b) is not administering the compound for about 3-120 days.

66. The method of claim 63 wherein step (b) is not administering the compound for about 3-120 days.

67. The method of claim 62 wherein step (b) comprises not administering the compound for about 4-60 days.

68. The method of claim 63 wherein step (b) comprises not administering the compound for about 4-60 days.

69. The method of claim 61 wherein the compound is 16 α -bromo-3 β -hydroxy-5 α -androst-17-one.

70. The method of claim 69 wherein the 16 α -bromo-3 β -hydroxy-5 α -androst-17-one comprises 16 α -bromo-3 β -hydroxy-5 α -androst-17-one hemihydrate.

71. The method of claim 66 wherein the compound is 16 α -bromo-3 β -hydroxy-5 α -androst-17-one.

72. The method of claim 71 wherein the 16 α -bromo-3 β -hydroxy-5 α -androst-17-one comprises 16 α -bromo-3 β -hydroxy-5 α -androst-17-one hemihydrate.

73. The method of claim 61 wherein the compound is 3 β ,16 α -dihydroxy-5 α -androst-17-one.

74. The method of claim 61 wherein the compound is 16 β -bromo-

75. The method of claim 61 wherein the compound is 3 β ,17 β -dihydroxyandrost-5-ene.

76. The method of claim 61 wherein the compound is 3 β ,7 β ,17 β -trihydroxyandrost-5-ene.

77. The method of claim 58 wherein the compound is 3 α ,17 β -dihydroxy-16 α -fluoroandrost-5-ene.

78. A method to treat a subject having, or subject to developing, diabetes, hyperglycemia or a hyperlipidemia, comprising administering to the subject an effective amount of a compound having the structure ##STR122## wherein R¹ is --OH, .dbd.O, --OR^{PR}, --SH, --SR^{PR}, --N₃, --NH₂, --N(R^{PR})₂, --O--Si--(R¹³)₃, --CN, --NO₂, --C(O)--CH₃, --F, --Cl, --Br, --I, an ester, a thioester, a phosphoester, a phosphothioester, a phosphonoester, a phosphiniester, a sulfite ester, a sulfate ester, an amide, an amino acid, a peptide, an ether, a thioether, an acyl group, a thioacyl group, a carbonate, a carbamate, a thioacetal, an optionally substituted heteroaryl moiety, an optionally substituted monosaccharide, an optionally substituted oligosaccharide, a nucleoside, a nucleotide, an **oligonucleotide** or a polymer; R² is --H, --OH, --OR^{PR}, .dbd.O, --SH, --SR^{PR}, .dbd.S, .dbd.CH₂, --N₃, --NH₂, --N(R^{PR})₂, --O--Si--(R¹³)₃, --CN, --NO₂, .dbd.NOH, .dbd.NOC(O)CH₃, --C(O)--CH₃, --F, --Cl, --Br, --I, an ester, a thioester, a phosphoester, a phosphothioester, a phosphonoester, a phosphiniester, a sulfite ester, a sulfate ester, an amide, an amino acid, a peptide, an ether, a thioether, an acyl group, a thioacyl group, a carbonate, a carbamate, a thioacetal, an optionally substituted alkenyl group, an optionally substituted alkynyl group, an optionally substituted aryl moiety, an optionally substituted heteroaryl moiety, an optionally substituted monosaccharide, an optionally substituted oligosaccharide, a nucleoside, a nucleotide, an **oligonucleotide** or a polymer; R³ is --OH, OR^{PR}, --SH, --SR^{PR}, .dbd.S, .dbd.CH₂, --N₃, --NH₂, --N(R^{PR})₂, --O--Si--(R¹³)₃, --CN, --NO₂, .dbd.NOH, .dbd.NOC(O)CH₃, --C(O)--CH₃, --F, --Cl, --Br, --I, an ester, a thioester, a phosphoester, a phosphothioester, a phosphonoester, a phosphiniester, a sulfite ester, a sulfate ester, an amide, an amino acid, a peptide, an ether, a thioether, an acyl group, a thioacyl group, a carbonate, a carbamate, a thioacetal, an optionally substituted alkyl group, an optionally substituted alkenyl group, an optionally substituted alkynyl group, an optionally substituted aryl moiety, an optionally substituted heteroaryl moiety, an optionally substituted monosaccharide, an optionally substituted oligosaccharide, a nucleoside, a nucleotide, an **oligonucleotide** or a polymer; R⁴ is --OH, --OR^{PR}, .dbd.O, --SH, --SR^{PR}, .dbd.S, .dbd.CH₂, .dbd.CH(CH₂)₀₋₁₅CH₃, --N₃, --NH₂, --N(R^{PR})₂, --O--Si--(R¹³)₃, --CN, --NO₂, .dbd.NOH, .dbd.NOC(O)CH₃, --C(O)--CH₃, --F, --Cl, --Br, --I, an ester, a thioester, a phosphoester, a phosphothioester, a phosphonoester, a phosphiniester, a sulfite ester, a sulfate ester, an amide, an amino acid, a peptide, an ether, a thioether, an acyl group, a thioacyl group, a carbonate, a carbamate, a thioacetal, an optionally substituted alkyl group, an optionally substituted alkenyl group, an optionally substituted alkynyl group, an optionally substituted aryl moiety, an optionally substituted heteroaryl moiety, an optionally substituted monosaccharide, an optionally substituted oligosaccharide, a nucleoside, a nucleotide, an **oligonucleotide** or a polymer, or R³ and both R⁴ together comprise a structure of formula 2 ##STR123## R⁵ and R⁶ independently are --H, --OH, --OR^{PR}, --SH, --SR^{PR}, --N₃, --NH₂, --N(R^{PR})₂, --O--Si--(R¹³)₃, --CN, --NO₂, --C(O)--CH₃, --F, --Cl, --Br, --I, an ester, a thioester, a phosphoester, a phosphothioester, a phosphonoester, a phosphiniester, a sulfite ester, a sulfate ester, an amide, an amino acid, a peptide, an ether, a thioether, an acyl group, a thioacyl group, a carbonate, a carbamate, a thioacetal, an optionally substituted alkyl group, an optionally substituted alkenyl group, an optionally substituted alkynyl group, an optionally substituted aryl moiety, an optionally substituted heteroaryl moiety, an optionally substituted monosaccharide, an optionally substituted oligosaccharide, a nucleoside, a nucleotide, an **oligonucleotide** or a polymer; R⁷ is --CHR¹⁰, --CHR¹⁰--CHR¹⁰--, --CHR¹⁰--CHR¹⁰--CHR¹⁰--, --CHR¹⁰--O--CHR¹⁰--, --CHR¹⁰--S--CHR¹⁰--, --CHR¹⁰--NR^{PR}--CHR¹⁰--, --O--, --O--CHR¹⁰--, --S--, --S--CHR¹⁰--, --NR^{PR}-- or --NR^{PR}--CHR¹⁰--, wherein R¹⁰ independently are --H, --OH, --OR^{PR}, .dbd.O, --SH,

--N(R^{PR})₂, --O--Si--(R¹³)₃, --CN, --NO₂,
 .dbd.NOH, .dbd.NOC(O)CH₃, --C(O)--CH₃, --F, --Cl, --Br, --I,
 an ester, a thioester, a phosphoester, a phosphothioester, a phosphonoester, a phosphiniester, a sulfite ester, a sulfate ester, an amide, an amino acid, a peptide, an ether, a thioether, an acyl group, a thioacyl group, a carbonate, a carbamate, a thioacetal, an optionally substituted alkyl group, an optionally substituted alkenyl group, an optionally substituted alkynyl group, an optionally substituted aryl moiety, an optionally substituted heteroaryl moiety, an optionally substituted monosaccharide, an optionally substituted oligosaccharide, a nucleoside, a nucleotide, an **oligonucleotide** or a polymer; R⁸ is --CHR¹⁰--, --CHR¹⁰--CHR¹⁰--, --O--, --O--CHR¹⁰--, --S--, --S--CHR¹⁰--, --NR^{PR}-- or --NR^{PR}--CHR¹⁰--, or R⁸ is absent, leaving a 5-membered ring, wherein R¹⁰ independently are --H, --OH, --OR^{PR}, .dbd.O, --SH, --SR^{PR}, S, .dbd.CH₂, --N₃, --NH₂, --N(R^{PR})₂, --O--Si--(R¹³)₃, --CN, --NO₂, .dbd.NOH, .dbd.NOC(O)CH₃, --C(O)--CH₃, --F, --Cl, --Br, --I, an ester, a thioester, a phosphoester, a phosphothioester, a phosphonoester, a phosphiniester, a sulfite ester, a sulfate ester, an amide, an amino acid, a peptide, an ether, a thioether, an acyl group, a thioacyl group, a carbonate, a carbamate, a thioacetal, an optionally substituted alkyl group, an optionally substituted alkenyl group, an optionally substituted alkynyl group, an optionally substituted aryl moiety, an optionally substituted heteroaryl moiety, an optionally substituted monosaccharide, an optionally substituted oligosaccharide, a nucleoside, a nucleotide, an **oligonucleotide** or a polymer; R⁹ is --CHR¹⁰--, --CHR¹⁰--CHR¹⁰--, --O--, --O--CHR¹⁰--, --S--, --S--CHR¹⁰--, --NR^{PR}-- or --NR^{PR}--CHR¹⁰--, or R⁹ is absent, leaving a 5-membered ring, wherein R¹⁰ independently are --H, --SH, --SR^{PR}, .dbd.S, .dbd.CH₂, --N₃, --NH₂, --N(R^{PR})₂, --O--Si--(R¹³)₃, --CN, --NO₂, .dbd.NOH, .dbd.NOC(O)CH₃, --C(O)--CH₃, --F, --Cl, --Br, --I, a thioester, an amide, an amino acid, a peptide, a thioether, an acyl group, a thioacyl group, a carbonate, a carbamate, a thioacetal, an optionally substituted alkyl group, an optionally substituted alkenyl group, an optionally substituted alkynyl group, an optionally substituted aryl moiety, an optionally substituted heteroaryl moiety, an optionally substituted monosaccharide, an optionally substituted oligosaccharide, a nucleoside, a nucleotide, an **oligonucleotide** or a polymer; D is a heterocycle or a 4-, 5-, 6- or 7-membered ring that comprises saturated carbon atoms, wherein 1, 2 or 3 ring carbon atoms of the 4-, 5-, 6- or 7-membered ring are optionally independently substituted with --O--, --S-- or --NR^{PR}-- or where 1, 2 or 3 hydrogen atoms of the heterocycle or 1 or 2 hydrogen atoms of the 4-, 5-, 6- or 7-membered ring are substituted with --OR^{PR}, --SR^{PR}, N(R^{PR})₂, --O--Si--(R¹³)₃, --CN, --NO₂, an ester, a thioester, a phosphoester, a phosphothioester, a phosphonoester, a phosphiniester, a sulfite ester, a sulfate ester, an amide, an amino acid, a peptide, an ether, a thioether, an acyl group, a thioacyl group, a carbonate, a carbamate, a thioacetal, a halogen, an optionally substituted alkyl group, an optionally substituted alkenyl group, an optionally substituted alkynyl group, an optionally substituted aryl moiety, an optionally substituted heteroaryl moiety, an optionally substituted monosaccharide, an optionally substituted oligosaccharide, a nucleoside, a nucleotide, an **oligonucleotide** or a polymer, or, one more of the ring carbons are substituted with .dbd.O or .dbd.S, or D comprises two 5- or 6-membered rings, wherein the rings are fused or are linked by 1 or 2 bonds; R^{PR} is a protecting group; R¹³ independently are C1-C6 alkyl.

79. The method of claim 78 wherein the compound is 3 α ,17 β -dihydroxy-16 α -fluoroandrost-5-ene, 3 α -hydroxy-16 α -fluoroandrost-5-ene-17-one, 3 α ,17 β -dihydroxy-16 α -fluoroandrost-4-ene or 3 α -hydroxy-16 α -fluoroandrost-4-ene-17-one.

80. The method of claim 78 wherein the level or activity of PPAR α , LXR α or SF-1 is modulated in the subject.

81. The method of claim 78 wherein the hyperlipidemia is hypercholesterolemia.

82. A method to treat a subject having, or subject to developing, diabetes, hyperglycemia or a hyperlipidemia, comprising administering to the subject an effective amount of a compound having the structure ##STR124## wherein R² is --OH, --OR^{PR}, .dbd.O, --SH, --SR^{PR}, .dbd.S, .dbd.CH₂, --N₃, --NH₂, --N(R^{PR})₂, --O--Si--(R¹³)₃, --CN, --NO₂, .dbd.NOH, .dbd.NOC(O)CH₃, --C(O)--CH₃, --F, --Cl, --Br, --I, an ester, a

phosphiniester, a sulfite ester, a sulfate ester, an amide, an amino acid, a peptide, an ether, a thioether, an acyl group, a thioacyl group, a carbonate, a carbamate, a thioacetal, an optionally substituted alkenyl group, an optionally substituted alkynyl group, an optionally substituted aryl moiety, an optionally substituted heteroaryl moiety, an optionally substituted monosaccharide, a nucleoside, an **oligonucleotide** or a polymer; R³ is --OH, --OR^{PR}, --SH, --SR^{PR}, .dbd.S, .dbd.CH₂, --N₃, --NH₂, --N(R^{PR})₂, --O--Si--(R¹³)₃, --CN, --NO₂, .dbd.NOH, .dbd.NOC(O)CH₃, --C(O)--CH₃, --F, --Cl, --Br, --I, an ester, a thioester, a phosphoester, a phosphothioester, a phosphonoester, a phosphiniester, a sulfite ester, a sulfate ester, an amide, an amino acid, a peptide, an ether, a thioether, an acyl group, a thioacyl group, a carbonate, a carbamate, a thioacetal, an optionally substituted alkyl group, an optionally substituted alkenyl group, an optionally substituted alkynyl group, an optionally substituted aryl moiety, an optionally substituted heteroaryl moiety, an optionally substituted monosaccharide, a nucleoside, a nucleotide, an **oligonucleotide** or a polymer; R⁴ is --OH, --OR^{PR}, .dbd.O, --SH, --SR^{PR}, .dbd.S, .dbd.CH₂, .dbd.CH(CH₂)₀₋₁₅CH₃, --N₃, --NH₂, --N(R^{PR})₂, --O--Si--(R¹³)₃, --CN, --NO₂, .dbd.NOH, .dbd.NOC(O)CH₃, --C(O)--CH₃, --F, --Cl, --Br, --I, an ester, a thioester, a phosphoester, a phosphothioester, a phosphonoester, a phosphiniester, a sulfite ester, a sulfate ester, an amide, an amino acid, a peptide, an ether, a thioether, an acyl group, a thioacyl group, a carbonate, a carbamate, a thioacetal, an optionally substituted alkyl group, an optionally substituted alkenyl group, an optionally substituted alkynyl group, an optionally substituted aryl moiety, an optionally substituted heteroaryl moiety, an optionally substituted monosaccharide, a nucleoside, a nucleotide, an **oligonucleotide** or a polymer, or R³ and both R⁴ together comprise a structure of formula 2 ##STR125## R⁵ and R⁶ independently are --H, --OH, --OR^{PR}, --SH, --SR^{PR}, --N₃, --NH₂, --N(R^{PR})₂, --O--Si--(R¹³)₃, --CN, --CH₃, --NO₂, --C(O)--CH₃, --F, --Cl, --Br, --I, an ester, a thioester, a phosphoester, a phosphothioester, a phosphonoester, a phosphiniester, a sulfite ester, a sulfate ester, an amide, an amino acid, a peptide, an ether, a thioether, an acyl group, a thioacyl group, a carbonate, a carbamate, a thioacetal, an optionally substituted alkyl group, an optionally substituted alkenyl group, an optionally substituted alkynyl group, an optionally substituted aryl moiety, an optionally substituted heteroaryl moiety, an optionally substituted monosaccharide, a nucleoside, a nucleotide, an **oligonucleotide** or a polymer, or, R is --CHR¹⁰--, --CHR¹⁰--CHR¹⁰--, --CHR¹⁰--CHR¹⁰--CHR¹⁰--, --CHR¹⁰--O--CHR¹⁰--, --CHR¹⁰--NR^{PR}--CHR¹⁰--, --O--, --O--CHR¹⁰--, --S--, --S--CHR¹⁰--, --NR^{PR}-- or --NR^{PR}--CHR¹⁰--, wherein R¹⁰ independently are --H, --OH, --OR^{PR}, .dbd.O, --SH, --SR^{PR}, .dbd.S, .dbd.CH₂, --N₃, --NH₂, --N(R^{PR})₂, --O--Si--(R¹³)₃, --CN, --NO₂, .dbd.NOH, .dbd.NOC(O)CH₃, --C(O)--CH₃, --F, --Cl, --Br, --I, an ester, a thioester, a phosphoester, a phosphothioester, a phosphonoester, a phosphiniester, a sulfite ester, a sulfate ester, an amide, an amino acid, a peptide, an ether, a thioether, an acyl group, a thioacyl group, a carbonate, a carbamate, a thioacetal, an optionally substituted alkyl group, an optionally substituted alkenyl group, an optionally substituted alkynyl group, an optionally substituted aryl moiety, an optionally substituted heteroaryl moiety, an optionally substituted monosaccharide, a nucleoside, a nucleotide, an **oligonucleotide** or a polymer; R⁸ is --CHR¹⁰--, --CHR¹⁰--CHR¹⁰--, --O--, --O--CHR¹⁰--, --S--, --S--CHR¹⁰--, --NR^{PR}-- or --NR^{PR}--CHR¹⁰--, or R⁹ is absent, leaving a 5-membered ring, wherein R¹⁰ independently are --H, --OH, --OR^{PR}, .dbd.O, --SH, --SR^{PR}, .dbd.S, .dbd.CH₂, --N₃, --NH₂, --N(R^{PR})₂, --O--Si--(R¹³)₃, --CN, --NO₂, .dbd.NOH, .dbd.NOC(O)CH₃, --C(O)--CH₃, --F, --Cl, --Br, --I, an ester, a thioester, a phosphoester, a phosphothioester, a phosphonoester, a phosphiniester, a sulfite ester, a sulfate ester, an amide, an amino acid, a peptide, an ether, a thioether, an acyl group, a thioacyl group, a carbonate, a carbamate, a thioacetal, an optionally substituted alkyl group, an optionally substituted alkenyl group, an optionally substituted alkynyl group, an optionally substituted aryl moiety, an optionally substituted heteroaryl moiety, an optionally substituted monosaccharide, a nucleoside, a nucleotide, an **oligonucleotide** or a polymer; R⁹ is --CHR¹⁰--, --CHR¹⁰--CHR¹⁰--, --O--, --O--CHR¹⁰--, --S--, --S--CHR¹⁰--, --NR^{PR}-- or --NR^{PR}--CHR¹⁰--, or

independently are --H, --SH, --SR^{PR}, .dbd.S, .dbd.CH₂,
 --N₃, --NH₂, --N(R^{PR})₂, --O--Si--(R¹³)₃,
 --CN, --NO₂, .dbd.NOH, .dbd.NOC(O)CH₃, --C(O)--CH₃, --F,
 --Cl, --Br, --I, a thioester, an amide, an amino acid, a peptide, a
 thioether, an acyl group, a thioacyl group, a carbonate, a carbamate, a
 thioacetal, an optionally substituted alkenyl group, an optionally
 substituted alkynyl group, an optionally substituted aryl moiety, an
 optionally substituted heteroaryl moiety, an optionally substituted
 monosaccharide, an optionally substituted oligosaccharide, a nucleoside,
 a nucleotide, an **oligonucleotide** or a polymer; D is a heterocycle or
 a 4-, 5-, 6- or 7-membered ring that comprises saturated carbon atoms,
 wherein 1, 2 or 3 ring carbon atoms of the 4-, 5-, 6- or 7-membered ring
 are optionally independently substituted with --O--, --S-- or
 --NR^{PR}-- or where 1, 2 or 3 hydrogen atoms of the heterocycle or 1
 or 2 hydrogen atoms of the 4-, 5-, 6- or 7-membered ring are substituted
 with --OR^{PR}, --SR^{PR}, --N(R^{PR})₂, --O--Si--
 (R¹³)₃, --CN, --NO₂, an ester, a thioester, a
 phosphoester, a phosphothioester, a phosphonoester, a phosphiniester, a
 sulfite ester, a sulfate ester, an amide, an amino acid, a peptide, an
 ether, a thioether, an acyl group, a thioacyl group, a carbonate, a
 carbamate, a thioacetal, a halogen, an optionally substituted alkyl
 group, an optionally substituted alkenyl group, an optionally
 substituted alkynyl group, an optionally substituted aryl moiety, an
 optionally substituted heteroaryl moiety, an optionally substituted
 monosaccharide, an optionally substituted oligosaccharide, a nucleoside,
 a nucleotide, an **oligonucleotide** or a polymer, or, one more of the
 ring carbons are substituted with .dbd.O or .dbd.S, or D comprises two
 5- or 6-membered rings, wherein the rings are fused or are linked by 1
 or 2 bonds, provided that the compound is not 3 β -hydroxyandrost-5-
 ene-17-one, 3 β -hydroxyandrost-5-ene-17-one 3-sulfate or an ester
 or ether derivative of either compound; R^{PR} is a protecting group;
 R¹³ independently are C1-C6 alkyl.

83. The method of claim 82 wherein the compound is 7 α ,17 β -
 dihydroxy-16 α -fluoroandrost-5-ene, 7 α -hydroxy-16 α -
 fluoroandrost-5-ene-17-one, 7 β ,17 β -dihydroxy-16 α -
 fluoroandrost-4-ene or 7 β -hydroxy-16 α -fluoroandrost-4-ene-17-
 one.

84. The method of claim 82 wherein the level or activity of PPAR α ,
 LXR α or SF-1 is modulated in the subject.

85. The method of claim 82 wherein the hyperlipidemia is
 hypercholesterolemia.

86. A method to treat or prevent an infection in a subject in need
 thereof, wherein the method comprises administering to the subject an
 effective amount of a formulation comprising (1) one or more excipients
 and (2) a compound(s) selected from the group consisting of (i)
 16 α -bromo-3 β -hydroxy-5 α -androstan-17-one, (ii)
 16 β -bromo-3 β -hydroxy-5 α -androstan-17-one, (iii)
 16 α -bromo-3 β -hydroxy-5 α -androstan-17-one and
 16 β -bromo-3 β -hydroxy-5 α -androstan-17-one and (iv)
 3 β ,16 α -dihydroxy-5 α -androstan-17-one, and wherein the
 infection is selected from the group consisting of a Plasmodium
 infection, a Trypanosoma infection, a togavirus infection, a flavivirus
 infection, a hepadnavirus infection, a papillomavirus infection, a
 Candida infection, a Mycoplasma infection, a Cryptosporidium infection
 or a Toxoplasma infection.

87. The method of claim 86 wherein the pathogen infection is a togavirus
 infection, a flavivirus infection or a hepadnavirus infection.

88. The method of claim 87 wherein the togavirus infection, flavivirus
 infection or hepadnavirus infection is a hepatitis C virus infection or
 a hepatitis B virus infection.

89. The method of claim 29 wherein the pathogen infection is a
 Plasmodium infection.

90. A liquid formulation that contains less than about 3% v/v water and
 comprises (1) 16 α -bromo-3 β -hydroxy-5 α -androstan-17-one
 and/or 16 β -bromo-3 β -hydroxy-5 α -androstan-17-one and (2)
 liquid excipients selected from the group consisting of (i) propylene
 glycol, a polyethylene glycol and ethanol, (ii) propylene glycol, a
 polyethylene glycol and benzyl alcohol, (iii) propylene glycol and a
 polyethylene glycol and (iv) propylene glycol, a polyethylene glycol and
 benzyl benzoate.

than about 0.3% v/v water.

92. The formulation of claim 90 wherein the formulation contains about 0.5-100 mg/mL of 16 α -bromo-3 β -hydroxy-5 α -androstan-17-one.

93. The formulation of claim 92 wherein the formulation comprises less than about 0.3% v/v water.

94. The formulation of claim 89 wherein the formulation further comprises a local anaesthetic.

95. A product produced by the process of contacting a composition comprising 16 α -bromo-3 β -hydroxy-5 α -androstan-17-one and/or 16 β -bromo-3 β -hydroxy-5 α -androstan-17-one and two liquid excipients with a third liquid excipient wherein the product comprises less than about 3% v/v water.

96. The product of claim 95 wherein the product comprises less than about 0.3% v/v water.

97. The product of claim 95 wherein the two liquid excipients are selected from a polyethylene glycol, propylene glycol, benzyl benzoate and an alcohol selected from the group consisting of benzyl alcohol and ethanol.

98. The product of claim 95 wherein the third liquid excipient is a polyethylene glycol, propylene glycol, benzyl benzoate or ethanol.

99. A product produced by the process of contacting a composition comprising 16 α -bromo-3 β -hydroxy-5 α -androstan-17-one or 16 β -bromo-3 β -hydroxy-5 α -androstan-17-one and three liquid excipients with a fourth liquid excipient wherein the product comprises less than about 3% v/v water.

100. The product of claim 99 wherein the product comprises less than about 0.3% v/v water.

101. The product of claim 99 wherein the three liquid excipients are selected from a polyethylene glycol, propylene glycol, benzyl benzoate and an alcohol selected from the group consisting of benzyl alcohol and ethanol.

102. The product of claim 101 wherein the fourth liquid excipient is a polyethylene glycol, propylene glycol, benzyl benzoate, benzyl alcohol or ethanol.

103. The product of claim 95 wherein the product has been stored in a closed container at about 5-40° C. for about 30 minutes to about 2 years.

104. The product of claim 99 wherein the product has been stored in a closed container at about 5-40° C. for about 30 minutes to about 2 years.

105. A compound having the structure ##STR126## wherein the dotted lines are optional double bonds; R¹ is --H; R² is --OH, --OR^{PR}, .dbd.O, --SH, --SR^{PR}, .dbd.S, .dbd.CH₂, --N₃, --NH₂, --N(R^{PR})₂, --O--Si--(R¹³)₃, --CN, --NO₂, .dbd.NOH, .dbd.NOC(O)CH₃, --C(O)--CH₃, --F, --Cl, --Br, --I, an ester, a thioester, a phosphoester, a phosphothioester, a phosphonoester, a phosphiniester, a sulfite ester, a sulfate ester, an amide, an amino acid, a peptide, an ether, a thioether, an acyl group, a thioacyl group, a carbonate, a carbamate, a thioacetal, an optionally substituted alkenyl group, an optionally substituted alkynyl group, an optionally substituted aryl moiety, an optionally substituted heteroaryl moiety, an optionally substituted monosaccharide, an optionally substituted oligosaccharide, a nucleoside, a nucleotide, an **oligonucleotide** or a polymer; R³ is --OH, --OR^{PR}, --SH, --SR^{PR}, .dbd.S, .dbd.CH₂, --N₃, --NH₂, --N(R^{PR})₂, --O--Si--(R¹³)₃, --CN, --NO₂, .dbd.NOH, .dbd.NOC(O)CH₃, --C(O)--CH₃, --F, --Cl, --Br, --I, an ester, a thioester, a phosphoester, a phosphothioester, a phosphonoester, a phosphiniester, a sulfite ester, a sulfate ester, an amide, an amino acid, a peptide, an ether, a thioether, an acyl group, a thioacyl group, a carbonate, a carbamate, a thioacetal, an optionally substituted alkyl group, an optionally substituted alkenyl group, an optionally substituted alkynyl group, an optionally substituted aryl moiety, an optionally substituted heteroaryl moiety, an optionally substituted monosaccharide, an optionally substituted oligosaccharide, a nucleoside, a nucleotide, an **oligonucleotide** or a polymer; R⁴

$\text{.dbd.S, .dbd.CH}_2, \text{.dbd.CH(CH}_2\text{)}_{0-15}\text{CH}_3, \text{--N}_3,$
 $\text{--NH}_2, \text{--N(R}^{\text{PR}}\text{)}_2, \text{--O--Si--(R}^{13}\text{)}_3, \text{--CN,}$
 $\text{--NO}_2, \text{.dbd.NOH, .dbd.NOC(O)CH}_3, \text{--C(O)--CH}_3, \text{--F, --Cl,}$
 $\text{--Br, --I, an ester, a thioester, a phosphoester, a phosphothioester, a}$
 $\text{phosphonoester, a phosphiniester, a sulfite ester, a sulfate ester, an}$
 $\text{amide, an amino acid, a peptide, an ether, a thioether, an acyl group, a}$
 $\text{thioacyl group, a carbonate, a carbamate, a thioacetal, an optionally}$
 $\text{substituted alkyl group, an optionally substituted alkenyl group, an}$
 $\text{optionally substituted alkynyl group, an optionally substituted aryl}$
 $\text{moiety, an optionally substituted heteroaryl moiety, an optionally}$
 $\text{substituted monosaccharide, an optionally substituted oligosaccharide, a}$
 $\text{nucleoside, a nucleotide, an } \mathbf{oligonucleotide} \text{ or a polymer, provided}$
 $\text{that both R}^4 \text{ are not --H, or R}^3 \text{ and both R}^4 \text{ together}$
 $\text{comprise a structure of formula 2} \quad \mathbf{##STR127##} \text{ R}^5 \text{ and R}^6$
 $\text{independently are --H, --OH, --OR}^{\text{PR}}, \text{--SH, --SR}^{\text{PR}}, \text{--N}_3,$
 $\text{--NH}_2, \text{--N(R}^{\text{PR}}\text{)}_2, \text{--O--Si--(R}^{13}\text{)}_3, \text{--CN,}$
 $\text{--CH}_3, \text{--NO}_2, \text{--C(O)--CH}_3, \text{--F, --Cl, --Br, --I, an}$
 $\text{ester, a thioester, a phosphoester, a phosphothioester, a}$
 $\text{phosphonoester, a phosphiniester, a sulfite ester, a sulfate ester, an}$
 $\text{amide, an amino acid, a peptide, an ether, a thioether, an acyl group, a}$
 $\text{thioacyl group, a carbonate, a carbamate, a thioacetal, an optionally}$
 $\text{substituted alkyl group, an optionally substituted alkenyl group, an}$
 $\text{optionally substituted alkynyl group, an optionally substituted aryl}$
 $\text{moiety, an optionally substituted heteroaryl moiety, an optionally}$
 $\text{substituted monosaccharide, an optionally substituted oligosaccharide, a}$
 $\text{nucleoside, a nucleotide, an } \mathbf{oligonucleotide} \text{ or a polymer, or,}$
 $\text{R}^7 \text{ is --CHR}^{10}\text{--}, \text{--CHR}^{10}\text{--CHR}^{10}\text{--},$
 $\text{--CHR}^{10}\text{--CHR}^{10}\text{--CHR}^{10}\text{--}, \text{--CHR}^{10}\text{--O--CHR}^{10}\text{--},$
 $\text{--CHR}^{10}\text{--S--CHR}^{10}\text{--}, \text{--CHR}^{10}\text{--NR}^{\text{PR}}\text{--CHR}^{10}\text{--},$
 $\text{--O--}, \text{--O--CHR}^{10}\text{--}, \text{--S--}, \text{--S--CHR}^{10}\text{--}, \text{--NR}^{\text{PR}}\text{-- or}$
 $\text{--NR}^{\text{PR}}\text{--CHR}^{10}\text{--}, \text{wherein R}^{10} \text{ independently are --H, --OH,}$
 $\text{--OR}^{\text{PR}}, \text{.dbd.O, --SH, --SR}^{\text{PR}}, \text{.dbd.S, .dbd.CH}_2,$
 $\text{--N}_3, \text{--NH}_2, \text{--N(R}^{\text{PR}}\text{)}_2, \text{--O--Si--(R}^{13}\text{)}_3,$
 $\text{--CN, --NO}_2, \text{.dbd.NOH, .dbd.NOC(O)CH}_3, \text{--C(O)--CH}_3, \text{--F,}$
 $\text{--Cl, --Br, --I, an ester, a thioester, a phosphoester, a}$
 $\text{phosphothioester, a phosphonoester, a phosphiniester, a sulfite ester, a}$
 $\text{sulfate ester, an amide, an amino acid, a peptide, an ether, a}$
 $\text{thioether, an acyl group, a thioacyl group, a carbonate, a carbamate, a}$
 $\text{thioacetal, an optionally substituted alkyl group, an optionally}$
 $\text{substituted alkenyl group, an optionally substituted alkynyl group, an}$
 $\text{optionally substituted aryl moiety, an optionally substituted heteroaryl}$
 $\text{moiety, an optionally substituted monosaccharide, an optionally}$
 $\text{substituted oligosaccharide, a nucleoside, a nucleotide, an}$
 $\mathbf{oligonucleotide} \text{ or a polymer; R}^8 \text{ is --CHR}^{10}\text{--},$
 $\text{--CHR}^{10}\text{--CHR}^{10}\text{--}, \text{--O--}, \text{--O--CHR}^{10}\text{--}, \text{--S--},$
 $\text{--S--CHR}^{10}\text{--}, \text{NR}^{\text{PR}}\text{-- or --NR}^{\text{PR}}\text{--CHR}^{10}\text{--}, \text{or R}^8$
 $\text{is absent, leaving a 5-membered ring, wherein R}^{10} \text{ independently are}$
 $\text{--H, --OH, --OR}^{\text{PR}}, \text{.dbd.O, --SH, --SR}^{\text{PR}}, \text{.dbd.S,}$
 $\text{.dbd.CH}_2, \text{--N}_3, \text{--NH}_2, \text{--N(R}^{\text{PR}}\text{)}_2,$
 $\text{--O--Si--(R}^{13}\text{)}_3, \text{--CN, --NO}_2, \text{.dbd.NOH,}$
 $\text{.dbd.NOC(O)CH}_3, \text{--C(O)--CH}_3, \text{--F, --Cl, --Br, --I, an ester, a}$
 $\text{thioester, a phosphoester, a phosphothioester, a phosphonoester, a}$
 $\text{phosphiniester, a sulfite ester, a sulfate ester, an amide, an amino}$
 $\text{acid, a peptide, an ether, a thioether, an acyl group, a thioacyl group, a}$
 $\text{carbonate, a carbamate, a thioacetal, an optionally substituted alkyl}$
 $\text{group, an optionally substituted alkenyl group, an optionally}$
 $\text{substituted alkynyl group, an optionally substituted aryl moiety, an}$
 $\text{optionally substituted heteroaryl moiety, an optionally substituted}$
 $\text{monosaccharide, an optionally substituted oligosaccharide, a nucleoside,}$
 $\text{a nucleotide, an } \mathbf{oligonucleotide} \text{ or a polymer; R}^9 \text{ is}$
 $\text{--CHR}^{10}\text{--}, \text{--CHR}^{10}\text{--CHR}^{10}\text{--}, \text{--O--}, \text{--O--CHR}^{10}\text{--},$
 $\text{--S--}, \text{--S--CHR}^{10}\text{--}, \text{--NR}^{\text{PR}}\text{-- or --NR}^{\text{PR}}\text{--CHR}^{10}\text{--}, \text{or}$
 $\text{R}^9 \text{ is absent, leaving a 5-membered ring, wherein R}^{10}$
 $\text{independently are --H, --SH, --SR}^{\text{PR}}, \text{.dbd.S, .dbd.CH}_2,$
 $\text{--N}_3, \text{--NH}_2, \text{--N(R}^{\text{PR}}\text{)}_2, \text{--O--Si--(R}^{13}\text{)}_3,$
 $\text{--CN, --NO}_2, \text{.dbd.NOH, .dbd.NOC(O)CH}_3, \text{--C(O)--CH}_3, \text{--F,}$
 $\text{--Cl, --Br, --I, a thioester, an amide, an amino acid, a peptide, a}$
 $\text{thioether, an acyl group, a thioacyl group, a carbonate, a carbamate, a}$
 $\text{thioacetal, an optionally substituted alkenyl group, an optionally}$
 $\text{substituted alkynyl group, an optionally substituted aryl moiety, an}$
 $\text{optionally substituted heteroaryl moiety, an optionally substituted}$
 $\text{monosaccharide, an optionally substituted oligosaccharide, a nucleoside,}$
 $\text{a nucleotide, an } \mathbf{oligonucleotide} \text{ or a polymer; R}^{10} \text{ independently}$
 $\text{are --OH, --OR}^{\text{PR}}, \text{.dbd.O, --SH, --SR}^{\text{PR}}, \text{.dbd.S, .dbd.CH}_2,$
 $\text{--N}_3, \text{--NH}_2, \text{--N(R}^{\text{PR}}\text{)}_2, \text{--O--Si--(R}^{13}\text{)}_3,$
 $\text{--CN, --NO}_2, \text{.dbd.NOH, .dbd.NOC(O)CH}_3, \text{--C(O)--CH}_3, \text{--F,}$
 $\text{--Cl, --Br, --I, an ester, a thioester, a phosphoester, a}$
 $\text{phosphothioester, a phosphonoester, a phosphiniester, a sulfite ester, a}$
 $\text{sulfate ester, an amide, an amino acid, a peptide, an ether, a}$
 $\text{thioether, an acyl group, a thioacyl group, a carbonate, a carbamate, a}$
 $\text{thioacetal, an optionally substituted alkyl group, an optionally}$

optionally substituted aryl moiety, an optionally substituted heteroaryl moiety, an optionally substituted monosaccharide, an optionally substituted oligosaccharide, a nucleoside, a nucleotide, an **oligonucleotide** or a polymer; D is a heterocycle or a 4-, 5-, 6- or 7-membered ring that comprises saturated carbon atoms, wherein 1, 2 or 3 ring carbon atoms of the 4-, 5-, 6- or 7-membered ring are optionally independently substituted with --O--, --S-- or --NR^{RPR}-- or where 1, 2 or 3 hydrogen atoms of the heterocycle or 1 or 2 hydrogen atoms of the 4-, 5-, 6- or 7-membered ring are substituted with --OR^{RPR}--, --SR^{RPR}, N(R^{RPR})₂, --O--Si--(R¹³)₃, --CN, --NO₂, an ester, a thioester, a phosphoester, a phosphothioester, a phosphonoester, a phosphiniester, a sulfite ester, a sulfate ester, an amide, an amino acid, a peptide, an ether, a thioether, an acyl group, a thioacyl group, a carbonate, a carbamate, a thioacetal, a halogen, an optionally substituted alkyl group, an optionally substituted alkenyl group, an optionally substituted alkynyl group, an optionally substituted aryl moiety, an optionally substituted heteroaryl moiety, an optionally substituted monosaccharide, an optionally substituted oligosaccharide, a nucleoside, a nucleotide, an **oligonucleotide** or a polymer, or, one more of the ring carbons are substituted with .dbd.O or .dbd.S, or D comprises two 5- or 6-membered rings, wherein the rings are fused or are linked by 1 or 2 bonds, provided that the compound is not 3 β -hydroxyandrost-5-ene-17-one, 3 β -hydroxyandrost-5-ene-17-one 3-sulfate or an ester or ether derivative of either compound; R^{RPR} is a protecting group; R¹³ independently are C1-C6 alkyl.

106. The compound of claim 105 having the structure ##STR128##

107. The compound of claim 105 wherein the compound is
 7 β -hydroxy-16 α -fluoroandrost-5-ene-17-one,
 7 α -hydroxy-16 α -fluoroandrost-5-ene-17-one,
 16 α -fluoroandrost-5-ene-7,17-dione, 7 β ,17 β -dihydroxy-
 16 α -fluoroandrost-5-ene or 7 α ,17 β -dihydroxy-16 α -
 fluoroandrost-5-ene.

108. A formulation comprising one or more excipients and a compound of claim 105.

108. A formulation comprising one or more excipients and a compound of claim 106.

109. A formulation comprising one or more excipients and a compound of claim 107.

110. The method of claim 34 wherein the condition is an allergy or inflammation condition.

111. The method of claim 110, wherein the allergy or inflammation condition is allergic bronchopulmonary aspergillosis, atopic asthma, allergic respiratory disease, allergic rhinitis, atopic dermatitis, lung fibrosis, subepithelial fibrosis in airway hyperresponsiveness, chronic sinusitis, perennial allergic rhinitis, Crohn's disease, ulcerative colitis, inflammatory bowel disease, chronic diarrhea or fibrosing alveolitis.

110. The method of claim 34, wherein the condition is an autoimmune disease.

111. The method of claim 27, wherein the autoimmune disease is systemic lupus erythematosus, myasthenia gravis, Grave's disease, diabetes, rheumatoid arthritis or osteoarthritis.

112. A compound having the structure ##STR129## wherein, the dotted lines are optional double bonds; R¹ and R² independently are --OH, --OR^{RPR}, --SH, --SR^{RPR}, --O--Si--(R¹³)₃, an ester, a thioester, a phosphoester, a phosphothioester, a phosphonoester, a phosphiniester, a sulfite ester, a sulfate ester, an ether, a thioether, a carbonate, a carbamate, an optionally substituted monosaccharide or an optionally substituted oligosaccharide; R³ is --OH, --OR^{RPR}, --SH, --SR^{RPR}, --O--Si--(R¹³)₃, --F, --Cl, --Br, --I, an ester, a thioester, a phosphoester, a phosphothioester, a phosphonoester, a phosphiniester, a sulfite ester, a sulfate ester, an ether, a thioether, a carbonate, a carbamate, an optionally substituted monosaccharide or an optionally substituted oligosaccharide; each R⁴ independently is --OH, --OR^{RPR}, --SH, --SR^{RPR}, --O--Si--(R¹³)₃, an ester, a thioester, a phosphoester, a phosphothioester, a phosphonoester, a phosphiniester, a sulfite ester, a sulfate ester, an ether, a thioether, a carbonate, a carbamate, an optionally substituted monosaccharide or an optionally substituted oligosaccharide, provided that both R⁴ are not --H; R⁵ and R independently are --H, --OH, --OR^{RPR}, --SH,

--O--Si--(R¹³)₃, --CN, --NO₂, --C(O)--CH₃, --F,
 --Cl, --Br, --I, an ester, a thioester, a phosphoester, a
 phosphothioester, a phosphonoester, a phosphiniester, a sulfite ester, a
 sulfate ester, an amide, an amino acid, a peptide, an ether, a
 thioether, an acyl group, a thioacyl group, a carbonate, a carbamate, a
 thioacetal, an optionally substituted alkyl group, an optionally
 substituted alkenyl group, an optionally substituted alkynyl group, an
 optionally substituted aryl moiety, an optionally substituted heteroaryl
 moiety, an optionally substituted monosaccharide, an optionally
 substituted oligosaccharide, a nucleoside, a nucleotide, an
oligonucleotide or a polymer; R is --CHR¹⁰--,
 --CHR¹⁰--CHR¹⁰--, --CHR¹⁰--CHR¹⁰--CHR¹⁰--,
 --CHR¹⁰--O--CHR¹⁰--, --CHR¹⁰--S--CHR¹⁰--,
 --CHR¹⁰--NRPR--CHR¹⁰--, --O--, --O--CHR¹⁰--, --S--,
 --S--CHR¹⁰--, --NRPR-- or --NRPR--CHR¹⁰--; R⁸
 and R⁹ independently are --CHR¹⁰--, --CHR¹⁰--CHR¹⁰--
 , --O--, --O--CHR¹⁰--, --S--, --S--CHR¹⁰--, --NRPR-- or
 --NRPR--CHR¹⁰--, or R⁸ or R⁹ independently is
 absent, leaving a 5-membered ring; R¹⁰ independently are --H,
 --OH, .dbd.O, --ORPR, --SH, --SRPR, .dbd.S, .dbd.CH₂,
 --N₃, --NH₂, --N(RPR)₂, --O--Si--(R¹³)₃,
 --CN, --NO₂, .dbd.NOH, .dbd.NOC(O)CH₃, --C(O)--CH₃, --F,
 --Cl, --Br, --I, an ester, a thioester, a phosphoester, a
 phosphothioester, a phosphonoester, a phosphiniester, a sulfite ester, a
 sulfate ester, an amide, an amino acid, a peptide, an ether, a
 thioether, an acyl group, a thioacyl group, a carbonate, a carbamate, a
 thioacetal, an optionally substituted alkyl group, an optionally
 substituted alkenyl group, an optionally substituted alkynyl group, an
 optionally substituted aryl moiety, an optionally substituted heteroaryl
 moiety, an optionally substituted monosaccharide, an optionally
 substituted oligosaccharide, a nucleoside, a nucleotide, an
oligonucleotide or a polymer; R¹³ independently are C₁₆
 alkyl; and RPR independently are a protecting group.

113. A formulation comprising a compound of claim 105 and one or more excipients.

114. The formulation of claim 113 wherein the formulation is for buccal or sublingual administration to a human.

115. The formulation of claim 113 wherein the formulation is for parenteral or topical administration to a human.

116. A formulation comprising a compound of claim 112 and one or more excipients.

117. The formulation of claim 116 wherein the formulation is for buccal or sublingual administration to a human.

118. The formulation of claim 116 wherein the formulation is for parenteral or topical administration to a human.

119. The compound of claim 112 having the structure ##STR130## wherein, R⁴ is --OH, --ORPR, --SH, --SRPR, --O--Si--(R¹³)₃, an ester, a thioester, a phosphoester, a phosphothioester, a phosphonoester, a phosphiniester, a sulfite ester, a sulfate ester, an ether, a thioether, a carbonate, a carbamate, an optionally substituted monosaccharide or an optionally substituted oligosaccharide.

120. The compound of claim 119 wherein R¹, R², R³ and R⁴ are --OH.

121. The compound of claim 119 wherein R¹, R² and R⁴ are --OH and R³ is --F, --Cl, --Br or --I.

122. A formulation comprising a compound of claim 119 and one or more excipients.

123. The formulation of claim 122 wherein the formulation is for buccal or sublingual administration to a human.

124. The formulation of claim 122 wherein the formulation is for parenteral or topical administration to a human.

AB The invention provides methods of using protein trans-splicing for the production of bispecific molecule which has a first antigen recognition portion that binds a C3b-like receptor and a second antigen recognition portion that binds an antigenic molecule present in the circulatory system of a mammal. The invention also provides bispecific molecules produced by protein trans-splicing. The bispecific molecules of the invention can be used for the clearance of pathogenic antigenic molecules from the circulatory system of a mammal. The invention further provides methods of using protein trans-splicing for the production of polyclonal libraries of bispecific molecules, which comprise populations of bispecific molecules with different antigen recognition specificities. Such polyclonal libraries of bispecific molecules can be used for targeting multiple epitopes of a pathogenic antigenic molecule and/or multiple variants of a pathogenic antigenic molecule.

CLM What is claimed is:

1. A method of producing a bispecific molecule having a first antigen recognition portion that binds a C3b-like receptor and a second antigen recognition portion that binds a pathogenic antigenic molecule, comprising contacting an N-intein first antigen recognition portion and a C-intein second antigen recognition portion under conditions such that protein trans-splicing occurs, wherein said N-intein first antigen recognition portion comprises said first antigen recognition portion conjugated to the amino terminus of an N-intein of a split intein, and wherein said C-intein second antigen recognition portion comprises said second antigen recognition portion conjugated to the carboxy terminus of a C-intein of said split intein, wherein the amino acid residue immediately at the C-terminal side of the splice junction of said C-intein is an amino acid residue selected from the group consisting of cysteine, serine, and threonine.
2. A method of producing a bispecific molecule having a first antigen recognition portion that binds a C3b-like receptor and a second antigen recognition portion that binds a pathogenic antigenic molecule, comprising: (a) obtaining an N-intein first antigen recognition portion by conjugating said first antigen recognition portion to the amino terminus of a molecule comprising an N-intein of a split intein; (b) obtaining a C-intein second antigen recognition portion by conjugating said second antigen recognition portion to the carboxy terminus of a molecule comprising a C-intein of said split intein, wherein the amino acid residue immediately at the C-terminal side of the splice junction of said C-intein is an amino acid residue selected from the group consisting of cysteine, serine, and threonine; and (c) contacting said N-intein first antigen recognition portion and said C-intein second antigen recognition portion under conditions such that protein trans-splicing occurs, to produce said bispecific molecule.
3. The method of claim 1 or 2, wherein said split intein is a naturally occurring split intein.
4. The method of claim 3, wherein the sequence of the N-intein of said split intein is SEQ ID NO:1, and the sequence of the C-intein of said split intein is SEQ ID NO:2.
5. The method of claim 1 or 2, wherein said split intein is an engineered split intein generated by separating a naturally occurring non-split intein into an amino terminal fragment and a carboxy terminal fragment such that said amino terminal fragment and said carboxy terminal fragment can reconstitute and undergo trans-splicing.
6. The method of claim 5, wherein said engineered split intein is generated from the Mycobacterium tuberculosis RecA intein.
7. The method of claim 1 or 2, wherein said N-intein further comprises a sequence of 1 or more native proximal N-extein amino acid residue(s) attached to the amino terminus of said N-intein.
8. The method of claim 7, wherein said N-intein comprises a sequence of 3 to 5 native proximal N-extein amino acid residue(s) attached to the amino terminus of said N-intein.
9. The method of claim 1 or 2, wherein said C-intein further comprises an amino acid residue selected from the group consisting of cysteine, serine, and threonine at the carboxy terminus of the splice junction.
10. The method of claim 9, wherein said C-intein further comprises a sequence of 1 or more native proximal C-extein amino acid residue(s) attached to the carboxy terminus of said C-intein.
11. The method of claim 10, wherein said C-intein comprises a sequence of 3 to 5 more native proximal C-extein amino acid residue(s) attached

12. The method of claim 1 or 2, wherein said N-intein comprises an unnatural amino acid residue such that upon conversion of said unnatural amino acid residue into normal amino acid residues by a suitable means said N-intein becomes functional for trans-splicing.

13. The method of claim 12, wherein said unnatural amino acid residue is O-(2-nitrobenzyl)serine, and said suitable means comprises irradiating using light having a wavelength in the range of 300-350 nm.

14. The method of claim 1 or 2, wherein said first antigen recognition portion comprises an antigen binding domain that binds a C3b-like receptor on a mammalian blood cell and an effector domain that facilitates transfer of said pathogenic antigen molecule from said mammalian blood cell to a phagocytic cell.

15. The method of claim 1 or 2, wherein said first antigen recognition portion is an monoclonal antibody.

16. The method of claim 15, wherein said N-intein first antigen recognition portion is generated by conjugating the carboxy terminus of the heavy chain of said monoclonal antibody to said amino terminus of said N-intein.

17. The method of claim 15, wherein said N-intein first antigen recognition portion fragment is generated by conjugating the carboxy terminus of the light chain of said monoclonal antibody to the amino terminus of said N-intein.

18. The method of claim 1 or 2, wherein said first antigen recognition portion is an ScFv that binds a C3b-like receptor fused to the N-terminus of an immunoglobulin Fc domain.

19. The method of claim 1 or 2, wherein said second antigen recognition portion comprises a polypeptide.

20. The method of claim 1 or 2, wherein said second antigen recognition portion comprises a nucleic acid.

21. The method of claim 1 or 2, wherein said N-intein first antigen recognition portion is produced recombinantly.

22. The method of claim 1 or 2, wherein said C-intein second antigen recognition portion is produced recombinantly.

23. The method of claim 1 or 2, wherein said C-intein second antigen recognition portion is produced by peptide synthesis.

24. The method of claim 1 or 2, wherein N-intein first antigen recognition portion and said C-intein second antigen recognition portion are recombinantly co-expressed in a cell, and wherein said N-intein first antigen recognition portion and said C-intein second antigen recognition portion reconstitute and undergo trans-splicing in said cell to produce said bispecific molecule.

25. The method of claim 1 or 2, wherein said N-intein first antigen recognition portion is recombinantly expressed in a first cell and said C-intein second antigen recognition portion is recombinantly expressed in a second cell, and wherein said N-intein first antigen recognition portion and said C-intein second antigen recognition portion are mixed in vitro to produce said bispecific molecule.

26. The method of claim 25, wherein said N-intein first antigen recognition portion and said C-intein second antigen recognition portion are expressed by separate expression vectors.

27. The method of claim 25, wherein said N-intein first antigen recognition portion and said C-intein second antigen recognition portion are expressed by one expression vector.

28. The method of claim 15, wherein the heavy and light chains of said anti-CR1 mAb are recombinantly expressed using same vector.

29. The method of claim 15, wherein the heavy and light chains of said anti-CR1 mAb are recombinantly expressed using separate vectors.

30. The method of claim 29, wherein said heavy and light chains of said anti-CR1 mAb are recombinantly expressed in same cell.

31. The method of claim 29, wherein said heavy and light chains of said anti-CR1 mAb are recombinantly expressed in separate cells using separate vectors, and wherein said heavy and light chains are assembled

32. A method of producing a bispecific molecule having a first antigen recognition portion that binds a C3b-like receptor and a second antigen recognition portion that binds a pathogenic antigenic molecule, comprising (a) contacting an N-intein first antigen recognition portion and a C-intein streptavidin under conditions such that trans-splicing occurs to produce a first antigen recognition portion-streptavidin molecule, wherein said N-intein first antigen recognition portion comprises said first antigen recognition portion conjugated to the amino terminus of an N-intein of a split intein, and wherein said C-intein streptavidin comprises a core streptavidin conjugated to the carboxy terminus of a C-intein of said split intein, wherein the amino acid residue immediately at the C-terminal side of the splice junction of said C-intein is an amino acid residue selected from the group consisting of cysteine, serine, and threonine; and (b) contacting said first antigen recognition portion-streptavidin molecule and a biotinylated second antigen recognition portion under conditions conducive to binding between streptavidin and biotin, to produce said bispecific molecule.

33. A method of producing a bispecific molecule having a first antigen recognition portion that binds a C3b-like receptor and a second antigen recognition portion that binds a pathogenic antigenic molecule, comprising: (a) obtaining an N-intein first antigen recognition portion by conjugating said first antigen recognition portion to the amino terminus of an N-intein of a split intein; (b) obtaining a C-intein streptavidin by conjugating a core streptavidin to the carboxy terminus of the C-intein of said split intein, wherein the amino acid residue immediately at the C-terminal side of the splice junction of said C-intein is an amino acid residue selected from the group consisting of cysteine, serine, and threonine; (c) contacting said N-intein first antigen recognition portion and said C-intein streptavidin under conditions such that protein trans-splicing occurs, to produce a first antigen recognition portion-streptavidin molecule, wherein said first antigen recognition portion is conjugated to said streptavidin; (d) obtaining a biotinylated second antigen recognition portion; and (e) contacting said first antigen recognition portion-streptavidin molecule and said biotinylated second antigen recognition portion under conditions conducive to binding between streptavidin and biotin, to produce said bispecific molecule.

34. The method of claim 32 or 33, wherein said C-intein streptavidin is generated recombinantly.

35. The method of claim 34, further comprising in step (b) the step of dissociating multimers of C-intein streptavidin.

36. The method of claim 32 or 33, wherein said biotinylated second antigen recognition portion is a biotinylated monoclonal antibody.

37. The method of claim 32 or 33, wherein said biotinylated second antigen recognition portion is produced by biotinylating of an antibody.

38. The method of claim 36, wherein said biotinylated monoclonal antibody is biotinylated at a single site.

39. The method of claim 36, wherein said biotinylated monoclonal antibody is biotinylated at a site such that the antigen binding capability of the antigen recognition domain is not impaired.

40. The method of claim 32 or 33, wherein said biotinylated second antigen recognition portion is a biotinylated nucleic acid.

41. The method of claim 40, wherein said biotinylated nucleic acid is produced from a nucleic acid sample by (a) amplifying said nucleic acid using a **PCR primer** containing a restriction digestion site for a restriction endonuclease wherein said restriction endonuclease generates an overhanging terminus upon cleavage at said restriction digestion site, and said restriction endonuclease does not cleave said nucleic acid other than within the sequence of said **PCR primer**; (b) cleaving the **PCR** product containing said **PCR primer** by said restriction endonuclease to generate an overhanging terminus; and (c) adding a biotinylated nucleotide at said overhanging terminus using a DNA polymerase to produce said biotinylated nucleic acid.

42. A method of producing a polyclonal library of bispecific molecules, each member of said polyclonal library having a first antigen recognition portion that binds a C3b-like receptor and a second antigen recognition portion that binds a pathogenic antigenic molecule, comprising contacting a plurality of N-intein first antigen recognition portions and a plurality of different C-intein second antigen recognition portions under conditions such that protein trans-splicing

each of said plurality of N-intein first antigen recognition portions comprises said first antigen recognition portion conjugated to the amino terminus of an N-intein of a split intein, and wherein each of said plurality of C-intein second antigen recognition portions comprises a second antigen recognition portion having a different binding specificity conjugated to the carboxy terminus of a C-intein of said split intein, wherein the amino acid residue immediately at the C-terminal side of the splice junction of said C-intein is an amino acid residue selected from the group consisting of cysteine, serine, and threonine.

43. A method of producing a polyclonal library of bispecific molecules, each member of said polyclonal library having a first antigen recognition portion that binds a C3b-like receptor and a second antigen recognition portion that binds a pathogenic antigenic molecule, comprising: (a) obtaining a plurality of N-intein first antigen recognition portions by a method comprising conjugating each of a plurality of first antigen recognition portions to the amino terminus of an N-intein of a split intein; (b) selecting from a phage display library a plurality of phage that display antigen recognition polypeptides, each having a different respective binding specificity using affinity screening; (c) obtaining a plurality of nucleic acids encoding said plurality of antigen recognition polypeptides, respectively; (d) fusing each nucleic acid of said plurality of nucleic acids to the amino terminus of a C-intein of said split intein to obtain a plurality of nucleic acids encoding a plurality of C-intein antigen recognition portions, wherein the amino acid residue immediately at the C-terminal side of the splice junction of said C-intein is an amino acid residue selected from the group consisting of cysteine, serine, and threonine; (e) expressing said plurality of nucleic acids encoding said plurality of C-intein antigen recognition portion fragments in a host; (f) obtaining said plurality of C-intein antigen recognition portions from said host; and (g) contacting said plurality of C-intein antigen recognition portions and a plurality of said N-intein first antigen recognition portions under conditions such that protein trans-splicing occurs, to produce said polyclonal library of bispecific molecules.

44. A method of producing a polyclonal library of bispecific molecules, each member of said polyclonal library having a first antigen recognition portion that binds a C3b-like receptor and a second antigen recognition portion that binds a pathogenic antigenic molecule, comprising: (a) obtaining a plurality of N-intein first antigen recognition portions by a method comprising conjugating each of a plurality of first antigen recognition portions to the amino terminus of an N-intein of a split intein; (b) fusing the amino terminus of the nucleic acid encoding a polypeptide antibody in each member of said phage display library to the carboxy terminus of a C-intein of said split intein to obtain a phage display library displaying a repertoire of C-intein antigen recognition portions, wherein the amino acid residue immediately at the C-terminal side of the splice junction of said C-intein is an amino acid residue selected from the group consisting of cysteine, serine, and threonine; (c) selecting from said phage display library displaying a repertoire of C-intein antigen recognition portions a plurality of phage that display C-intein antigen recognition portions, each having a different respective binding specificity using affinity screening; (d) obtaining a plurality of nucleic acids encoding said plurality of C-intein antigen recognition portions, respectively; (e) expressing said plurality of nucleic acids encoding said plurality of C-intein antigen recognition portions in a host; (g) obtaining said plurality of C-intein antigen recognition portions from said host; and (h) contacting said plurality of C-intein antigen recognition portions and a plurality of said N-intein first antigen recognition portions under conditions such that protein trans-splicing occurs, to produce said polyclonal library of bispecific molecules.

45. A method of producing a polyclonal library of bispecific molecules, each member of said polyclonal library having a first antigen recognition portion that binds a C3b-like receptor and a second antigen recognition portion that binds a pathogenic antigenic molecule, comprising (a) contacting a population of N-intein first antigen recognition portions and a population of C-intein streptavidin under conditions such that protein trans-splicing occurs to produce a population of first antigen recognition portion-streptavidin molecules, wherein each of said plurality of N-intein first antigen recognition portions comprises said first antigen recognition portion conjugated to the amino terminus of an N-intein of a split intein, and wherein each of said plurality of C-intein streptavidin comprises a core streptavidin conjugated to the carboxy terminus of a C-intein of said split intein, wherein the amino acid residue immediately at the C-terminal side of the splice junction of said C-intein is an amino acid residue selected from the group consisting of cysteine, serine, and threonine; and (b) contacting said population of first antigen recognition portion

recognition portions, each having a different antigen recognition specificity under conditions conducive to binding between streptavidin and biotin, to produce said polyclonal library of bispecific molecules.

46. A method of producing a polyclonal library of bispecific molecules, each member of said polyclonal library having a first antigen recognition portion that binds a C3b-like receptor and a second antigen recognition portion that binds a pathogenic antigenic molecule, comprising: (a) obtaining a population of N-intein first antigen recognition portions by a method comprising conjugating each of a population of first antigen recognition portions to the amino terminus of an N-intein of a split intein; (b) obtaining a population of C-intein streptavidin by a method comprising conjugating each of a population of core streptavidins to a C-intein of said split intein, wherein the amino acid residue immediately at the C-terminal side of the splice junction of said C-intein is an amino acid residue selected from the group consisting of cysteine, serine, and threonine; (c) contacting said population of N-intein first antigen recognition portions with said population of C-intein streptavidins under conditions such that protein trans-splicing occurs to produce a population of first antigen recognition portion streptavidin molecules; (d) selecting from a phage display library a plurality of phage that display antigen recognition polypeptides, each having a different respective binding specificity using affinity screening; (e) obtaining a plurality of nucleic acids encoding said plurality of antigen recognition polypeptides, respectively; (f) expressing said plurality of nucleic acids encoding said plurality of antigen recognition polypeptides in a host; (g) obtaining said plurality of antigen recognition polypeptides from said host; (h) obtaining a plurality of biotinylated antigen recognition polypeptides by conjugating each member of said plurality of antigen recognition polypeptides with biotin using an activated biotin agent; and (i) contacting said plurality of biotinylated antigen recognition polypeptides and said population of first antigen recognition portion streptavidin molecules under conditions conducive to binding between streptavidin and biotin, to produce said polyclonal library of bispecific molecules.

47. The method of claim 42, 43, 44, 45, or 46, wherein said plurality of antigen recognition portions has binding specificities for a plurality of epitopes of an antigen.

48. The method of claim 42, 43, 44, 45, or 46, wherein said plurality of antigen recognition portions has binding specificities for a plurality of variants of an antigen.

49. The method of claim 43 or 46, wherein said plurality of nucleic acids encoding said plurality of antigen recognition polypeptides is fused to said C-intein without clonal isolation.

50. The method of claim 43 or 46, wherein step (a) further comprising obtaining a plurality of different N-intein first antigen recognition portions by conjugating each of a plurality of different first antigen recognition portions to the amino terminus of a plurality of N-inteins of said split intein.

51. A method of producing a C-intein antigen recognition portion phage display library, each phage in said C-intein antigen recognition portion phage display library displaying on its surface a different C-intein antigen recognition portion, comprising fusing the amino terminus of the nucleic acid encoding a polypeptide antibody in each of a plurality of members of a phage display library to the carboxy terminus of a C-intein of a split intein to obtain a phage display library displaying a repertoire of C-intein antigen recognition portions, wherein the amino acid residue immediately at the C-terminal side of the splice junction of said C-intein is an amino acid residue selected from the group consisting of cysteine, serine, and threonine.

52. A bispecific molecule, comprising: (a) a first antigen recognition portion that binds a C3b-like receptor; and (b) a second antigen recognition portion that binds a pathogenic antigenic molecule; wherein said first antigen recognition portion comprises a monoclonal antibody, and wherein said second antigen recognition portion is conjugated to a carboxy terminus of a heavy or light chain of said first antigen recognition portion.

53. A bispecific molecule, comprising: (a) a first antigen recognition portion that binds a C3b-like receptor; (b) a linker; and (c) a second antigen recognition portion that binds a pathogenic antigenic molecule; wherein said second antigen recognition portion is conjugated to a carboxy terminus of said first antigen recognition portion via said linker.

peptide linker.

55. The bispecific molecule of claim 53, wherein said linker is a streptavidin-biotin linker.

56. The bispecific molecule of claim 53, wherein said linker is a Poly(ethylene glycol) linker.

57. The bispecific molecule of claim 53, wherein said first antigen recognition portion comprises an antigen binding domain that binds a C3b-like receptor on a mammalian blood cell and an effector domain that facilitates transfer of said pathogenic antigenic molecule from said mammalian blood cell to a phagocytic cell.

58. The bispecific molecule of claim 53, wherein said first antigen recognition portion is a monoclonal antibody.

59. The bispecific molecule of claim 52 or 58, wherein said second antigen recognition portion is conjugated to the C-terminus of a heavy chain of said first antigen recognition portion.

60. The bispecific molecule of claim 52 or 58, wherein said second antigen recognition portion is conjugated to the C-terminus of a light chain of said first antigen recognition portion.

61. The bispecific molecule of claim 52 or 58, wherein said second antigen recognition portion comprises two antigen recognition moieties each conjugated to the C-terminus of a different heavy chain of said first antigen recognition portion.

62. The bispecific molecule of claim 52 or 58, wherein said first antigen recognition portion is an anti-CR1 monoclonal antibody produced by the hybridoma cell line ATCC HB 8592 from the American Type Culture Collection (ATCC), and wherein the second antigen recognition portion comprises the α -subunit of Fc ϵ RI receptor.

63. The bispecific molecule of claim 52 or 58, wherein said second antigen recognition portion comprises two antigen recognition moieties each conjugated to the C-terminus of a different light chain of said first antigen recognition portion.

64. The bispecific molecule of claim 61, wherein said two antigen recognition moieties are the same antigen recognition moieties.

65. The bispecific molecule of claim 61, wherein said two antigen recognition moieties are different antigen recognition moieties.

66. The bispecific molecule of claim 65, wherein said different antigen recognition moieties target said pathogenic antigen cooperatively.

67. The bispecific molecule of claim 65, wherein the action of one of said different antigen recognition moieties enhances the binding affinity of the other one of said different antigen recognition moieties.

68. The bispecific molecule of claim 52 or 53, wherein said second antigen recognition portion comprises a peptide or polypeptide.

69. The bispecific molecule of claim 68, wherein said peptide or polypeptide is an antibody.

70. The bispecific molecule of claim 69, wherein said peptide or polypeptide is a monoclonal antibody.

71. The bispecific molecule of claim 68, wherein said peptide or polypeptide comprises an epitope.

72. The bispecific molecule of claim 52 or 53, wherein said second antigen recognition portion comprises a nucleic acid.

73. A polyclonal library of bispecific molecules comprising a plurality of bispecific molecules each comprising (a) a first antigen recognition portion that binds a C3b-like receptor, and (b) a different second antigen recognition portion, said different second antigen recognition portions having different binding specificities, wherein said first antigen recognition portion comprises a monoclonal antibody, and wherein said second antigen recognition portion is conjugated to a carboxy terminus of said first antigen recognition portion.

74. A polyclonal library of bispecific molecules comprising a plurality of bispecific molecules, each comprising: (a) a first antigen recognition portion that binds a C3b-like receptor; (b) a linker; and

binding specificity; wherein said second antigen recognition portion is conjugated to a carboxy terminus of said first antigen recognition portion via said linker.

75. The bispecific molecule of claim 74, wherein said linker is a peptide linker.

76. The bispecific molecule of claim 74, wherein said linker is a streptavidin-biotin linker.

77. The bispecific molecule of claim 74, wherein said linker is a Poly (ethylene glycol) linker.

78. The polyclonal library of bispecific molecules of claim 74, wherein the first antigen recognition portion of each bispecific molecule comprises an antigen binding domain that binds a C3b-like receptor on mammalian blood cell and an effector domain that facilitates transfer of said pathogenic antigenic molecule from said mammalian blood cell to a phagocytic cell.

79. The polyclonal library of bispecific molecules of claim 74, wherein the first antigen recognition portion of each bispecific molecule is a monoclonal antibody.

80. The polyclonal library of bispecific molecules of claim 73 or 79, wherein for each bispecific molecule said second antigen recognition portion is conjugated to the C-terminus of a heavy chain of said first antigen recognition portion.

81. The polyclonal library of bispecific molecules of claim 73 or 79, wherein for each bispecific molecule said second antigen recognition portion is conjugated to the C-terminus of a light chain of said first antigen recognition portion.

82. The polyclonal library of bispecific molecules of claim 73 or 79, wherein for each bispecific molecule said second antigen recognition portion comprises two antigen recognition moieties each conjugated to the C-terminus of a different heavy chain of said first antigen recognition portion.

83. The polyclonal library of bispecific molecules of claim 73 or 79, wherein for each bispecific molecule said second antigen recognition portion comprises two antigen recognition moieties each conjugated to the C-terminus of a different light chain of said first antigen recognition portion.

84. The polyclonal library of bispecific molecules of claim 82, wherein said two antigen recognition moieties are same antigen recognition moieties.

85. The polyclonal library of bispecific molecules of claim 82, wherein said two antigen recognition moieties are different antigen recognition moieties.

86. The polyclonal library of bispecific molecules of claim 85, wherein for each bispecific molecule said different antigen recognition moieties target said pathogenic antigen cooperatively.

87. The polyclonal library of bispecific molecules of claim 85, wherein the action of one of said different antigen recognition moieties enhances the binding affinity of the other one of said different antigen recognition moieties.

88. The polyclonal library of bispecific molecules of claim 73 or 74, wherein for each bispecific molecule said second antigen recognition portion comprises a peptide or polypeptide.

89. The polyclonal library of bispecific molecules of claim 88, wherein for each bispecific molecule said peptide or polypeptide is an antibody.

90. The polyclonal library of bispecific molecules of claim 88, wherein said peptide or polypeptide is a monoclonal antibody.

91. The polyclonal library of bispecific molecules of claim 88, wherein said peptide or polypeptide comprises an epitope.

92. The polyclonal library of bispecific molecules of claim 73 or 74, wherein for each bispecific molecule said second antigen recognition portion comprises a nucleic acid.

93. A composition comprising a plurality of purified bispecific molecules, wherein each bispecific molecule of said plurality of

portion that binds a C3b-like receptor and a second antigen recognition portion that binds a pathogenic antigenic molecule, wherein said first antigen recognition portion comprises a monoclonal antibody, and wherein said second antigen recognition portion is conjugated to a carboxy terminus of a heavy or light chain of said first antigen recognition portion, said plurality of purified bispecific molecules each comprising a different second antigen recognition portions that has a different binding specificity.

94. A composition comprising a plurality of purified bispecific molecules, wherein each bispecific molecule of said plurality of purified bispecific molecules comprises: (a) a first antigen recognition portion that binds a C3b-like receptor; and (b) a second antigen recognition portion that binds a pathogenic antigenic molecule; wherein said first antigen recognition portion comprises a monoclonal antibody, and wherein said second antigen recognition portion is conjugated to a carboxy terminus of a heavy or light chain of said first antigen recognition portion, said plurality of purified bispecific molecules each comprising a different second antigen recognition portions that has a different binding specificity.

95. A molecule, comprising: (a) an antigen recognition portion that binds a C3b-like receptor; and (b) an N-intein moiety comprising an N-intein of a split intein; wherein said antigen recognition portion is conjugated to the amino terminal end of said N-intein moiety.

96. The molecule of claim 95, wherein said antigen recognition portion is conjugated to said N-intein via a linker.

97. The molecule of claim 95, wherein said N-intein moiety is an N-intein.

98. The molecule of claim 95 or 96, wherein said antigen recognition portion is a monoclonal antibody.

99. The molecule of claim 95 or 96, wherein said antigen recognition portion is an ScFv fused to an Fc domain.

100. The molecule of claim 95 or 96, wherein said antigen recognition portion is a chimeric antibody comprising variable domains or complementarity determining regions (CDRs) from one species and constant domain from another species.

101. The molecule of claim 100, wherein said variable domains or complementarity determining regions (CDRs) are murine and said constant domain is human.

102. The molecule of claim 95 or 96, wherein said N-intein further comprises a sequence of 1 or more native proximal N-extein amino acid residue.

103. The molecule of claim 102, wherein said N-intein comprises a sequence of 3 to 5 native proximal N-extein amino acid residue.

104. The molecule of claim 95 or 96, wherein said N-intein comprises at the N-terminus an O-(2-nitrobenzyl)serine.

105. The molecule of claim 95 or 96, wherein said N-intein is the N-intein of the split intein encoded in the DnaE gene of Ssp.

106. The molecule of claim 102, wherein said N-intein is the N-intein of the split intein encoded in the DnaE gene of Ssp.

107. The molecule of claim 105, wherein said antigen recognition portion is a monoclonal antibody as produced by the hybridoma cell line ATCC HB 8592 from the American Type Culture Collection (ATCC).

108. The molecule of claim 106, wherein said antigen recognition portion is a monoclonal antibody as produced by the hybridoma cell line ATCC HB 8592 from the American Type Culture Collection (ATCC).

109. The molecule of claim 95 or 96, wherein said N-intein is the N-intein of an engineered split intein generated from the Mtu RecA intein.

110. A molecule, comprising: (a) an antigen recognition portion; and (b) a C-intein moiety comprising a C-intein of a split intein; wherein said antigen recognition portion is conjugated to the carboxy terminal end of said C-intein moiety, and wherein the amino acid residue immediately at the C-terminal side of the splice junction of said C-intein is an amino acid residue selected from the group consisting of cysteine, serine, and threonine.

111. The molecule of claim 110, wherein said antigen recognition portion is conjugated to said C-intein via a linker.
112. The molecule of claim 110, wherein said C-intein moiety is a C-intein.
113. The molecule of claim 110 or 111, wherein said antigen recognition portion comprises a peptide or polypeptide.
114. The molecule of claim 110 or 111, wherein said antigen recognition portion comprises a nucleic acid.
115. The molecule of claim 110 or 111, wherein said C-intein further comprises, after said amino acid residue selected from the group consisting of cysteine, serine, and threonine, a sequence of 1 or more native proximal C-extein amino acid residues.
116. The molecule of claim 113, wherein said C-intein comprises, after said amino acid residue selected from the group consisting of cysteine, serine, and threonine, a sequence of 3 to 5 native proximal C-extein amino acid residues.
117. The molecule of claim 110 or 111, wherein said C-intein is the C-intein of the split intein encoded in the DnaE gene of Ssp.
118. The molecule of claim 115, wherein said C-intein is the C-intein of the split intein encoded in the DnaE gene of Ssp.
119. The C-intein antigen recognition portion of claim 117, wherein said antigen recognition portion comprises the α -subunit of the Fc ϵ RI receptor.
120. The molecule of claim 110 or 111, wherein said C-intein is the C-intein of an engineered split intein generated from the Mtu RecA intein.
121. The molecule of claim 115, wherein said C-intein is the C-intein of an engineered split intein generated from the Mtu RecA intein.
122. A molecule, comprising: (a) a streptavidin; and (b) a C-intein of a split intein comprising, immediately at the C-terminal side of the splice junction, an amino acid residue selected from the group consisting of cysteine, serine, and threonine; wherein said streptavidin is conjugated to the carboxy terminus of said C-intein.
123. The molecule of claim 122, wherein said streptavidin is conjugated to said C-intein via a linker.
124. The molecule of claim 122 or 123, wherein said C-intein further comprises a sequence of 1 or more native proximal C-extein amino acid residues.
125. The molecule of claim 124, wherein said C-intein further comprises a sequence of 3 to 5 native proximal C-extein amino acid residues.
126. The molecule of claim 122 or 123, wherein said C-intein is the C-intein of the split intein encoded in the DnaE gene of Ssp.
127. The molecule of claim 124, wherein said C-intein is the C-intein of the split intein encoded in the DnaE gene of Ssp.
128. The molecule of claim 122 or 123, wherein said C-intein is the C-intein of an engineered split intein generated from the Mtu RecA intein.
129. A C-intein antigen recognition portion phage display library, comprising a plurality of phage, each phage in said plurality displaying on its surface a different C-intein polypeptide fusion protein, wherein each polypeptide has a different binding specificity.
130. An expression vector, comprising a transcriptional regulatory element operably linked to a nucleotide sequence encoding a first antigen recognition portion that binds a C3b-like receptor fused to the amino terminus of the N-intein of a split intein.
131. An expression vector, comprising a transcriptional regulatory element operably linked to a nucleotide sequence encoding a second antigen recognition portion fused to the carboxy terminus of a C-intein of a split intein wherein the amino acid residue immediately at the C-terminal side of the splice junction of said C-intein is an amino acid residue selected from the group consisting of cysteine, serine, and threonine.

132. An expression vector, comprising a transcriptional regulatory element operably linked to a nucleotide sequence encoding a core streptavidin fused to the carboxy terminus of a C-intein of a split intein wherein the amino acid residue immediately at the C-terminal side of the splice junction of said C-intein is an amino acid residue selected from the group consisting of cysteine, serine, and threonine.

133. A nucleic acid encoding an N-intein fused to the carboxy terminus of a heavy chain of an anti-CR1 monoclonal antibody.

134. An expression vector, comprising a transcriptional regulatory element operably linked to a nucleotide sequence encoding an N-intein fused to the carboxy terminus of a heavy chain of an anti-CR1 monoclonal antibody.

135. A nucleic acid encoding an N-intein fused to the carboxy terminus of the light chain of an anti-CR1 monoclonal antibody.

136. An expression vector, comprising a transcriptional regulatory element operably linked to a nucleotide sequence encoding an N-intein fused to the carboxy terminus of a light chain of an anti-CR1 monoclonal antibody.

137. The nucleic acid of claim 133 or 135, wherein said N-intein is the N-intein of the split intein encoded in the DnaE gene of Ssp.

138. The nucleic acid of claim 133 or 135, wherein said N-intein is the N-intein of an engineered split intein generated from the Mtu RecA intein.

139. The nucleic acid of claim 133 or 135, wherein said anti-CR1 monoclonal antibody is as produced by the hybridoma cell line ATCC HB 8592 from the American Type Culture Collection (ATCC).

140. A nucleic acid encoding an N-intein fused to the carboxy terminus of the Fc domain that is fused to a scFv.

141. An expression vector, comprising a transcriptional regulatory element operably linked to a nucleotide sequence encoding an N-intein fused to the carboxy terminus of the Fc domain that is fused to a scFv.

142. A nucleic acid encoding a C-intein fused to the amino terminus of a polypeptide that binds an antigen.

143. An expression vector, comprising a transcriptional regulatory element operably linked to a nucleotide sequence encoding a C-intein fused to the amino terminus of polypeptide that binds an antigen.

144. A nucleic acid encoding a C-intein fused to the amino terminus of a core streptavidin via an optional linker.

145. An expression vector, comprising a transcriptional regulatory element operably linked to a nucleotide sequence encoding a C-intein fused to the amino terminus of core streptavidin via an optional linker.

146. A plurality of nucleic acids encoding a plurality of C-intein antigen recognition portions.

147. A plurality of expression vectors, each comprising a transcriptional regulatory element operably linked to a nucleotide sequence encoding a C-intein antigen recognition portion.

148. A kit comprising in a container a bispecific molecule having a first antigen recognition portion that binds a C3b-like receptor and a second antigen recognition portion that binds a pathogenic antigenic molecule, wherein said first antigen recognition portion comprises a monoclonal antibody, and wherein said second antigen recognition portion is conjugated to a carboxy terminus of a heavy or light chain of said first antigen recognition portion.

149. A kit comprising in a container a bispecific molecule comprising: (a) a first antigen recognition portion that binds a C3b-like receptor; (b) a linker; and (c) a second antigen recognition portion that binds a pathogenic antigenic molecule; wherein said second antigen recognition portion is conjugated to a carboxy terminus of said first antigen recognition portion via said linker.

150. A kit comprising in a container a molecule comprising: (a) an antigen recognition portion that binds a C3b-like receptor; and (b) an N-intein moiety comprising an N-intein of a split intein; wherein said antigen recognition portion is conjugated to the amino terminal end of said N-intein moiety.

151. A kit comprising in a container a molecule comprising: (a) an antigen recognition portion; and (b) a C-intein moiety comprising a C-intein of a split intein; wherein said antigen recognition portion is conjugated to the carboxy terminal end of said C-intein moiety, and wherein the amino acid residue immediately at the C-terminal side of the splice junction of said C-intein is an amino acid residue selected from the group consisting of cysteine, serine, and threonine.

152. A kit comprising in two containers a first molecule comprising an antigen recognition portion that binds a C3b-like receptor and an N-intein moiety comprising an N-intein of a split intein, wherein said antigen recognition portion is conjugated to the amino terminal end of said N-intein moiety; and a second molecule comprising an antigen recognition portion and a C-intein moiety comprising a C-intein of a split intein, wherein said antigen recognition portion is conjugated to the carboxy terminal end of said C-intein peptide, and wherein the amino acid residue immediately at the C-terminal side of the splice junction of said C-intein is an amino acid residue selected from the group consisting of cysteine, serine, and threonine.

153. A kit comprising in one or more containers a first vector and a second vector, said first vector comprising a first DNA sequence encoding a first antigen recognition portion that binds a C3b-like receptor fused to the amino terminus of an N-intein peptide comprising an N-intein of a split intein, said second vector comprising a second DNA sequence encoding a second antigen recognition portion fused to the carboxy terminus of a C-intein peptide comprising a C-intein of said split intein, wherein the amino acid residue immediately at the C-terminal side of the splice junction of said C-intein peptide is an amino acid residue selected from the group consisting of cysteine, serine, and threonine.

154. A kit comprising in one or more containers a first vector and a second vector, said first vector comprising a first DNA sequence encoding a first antigen recognition portion that binds a C3b-like receptor fused to the amino terminus of an N-intein peptide comprising an N-intein of a split intein, said second vector comprising a second DNA sequence encoding a core streptavidin fused to the carboxy terminus of a C-intein peptide comprising a C-intein of said split intein wherein the amino acid residue immediately at the C-terminal side of the splice junction of said C-intein peptide is an amino acid residue selected from the group consisting of cysteine, serine, and threonine, and in a separate container a biotinylated second antigen recognition portion.

155. A kit comprising a phage display library, wherein each phage displays a different C-intein polypeptide fusion protein on its surface.

156. The kit of claim 153 or 154, wherein said first vector further comprises a first promoter operably linked to said first DNA sequence, and said second vector further comprises a second promoter operably linked to said second DNA sequence.

157. A cell transformed with a vector comprising a DNA sequence encoding a first antigen recognition portion that binds a C3b-like receptor fused to the amino terminus of the N-intein of a split intein.

158. A cell transformed with a vector comprising a DNA sequence encoding a second antigen recognition portion fused to the carboxy terminus of a C-intein of a split intein wherein the amino acid residue immediately at the C-terminal side of the splice junction of said C-intein is an amino acid residue selected from the group consisting of cysteine, serine, and threonine.

159. A cell transformed with a vector comprising a DNA sequence encoding a core streptavidin fused to the carboxy terminus of a C-intein of a split intein wherein the amino acid residue immediately at the C-terminal side of the splice junction of said C-intein is an amino acid residue selected from the group consisting of cysteine, serine, and threonine.

160. A cell transformed with a first vector and a second vector, said first vector comprising a first DNA sequence encoding a first antigen recognition portion that binds a C3b-like receptor fused to the amino terminus of an N-intein of a split intein, said second vector comprising a second DNA sequence encoding a second antigen recognition portion fused to the carboxy terminus of a C-intein of said split intein wherein the amino acid residue immediately at the C-terminal side of the splice junction of said C-intein is an amino acid residue selected from the group consisting of cysteine, serine, and threonine.

161. A cell containing a first antigen recognition portion that binds a C3b-like receptor fused to the amino terminus of the N-intein of a split

162. A cell containing a second antigen recognition portion fused to the carboxy terminus of a C-intein of a split intein, wherein the amino acid residue immediately at the C-terminal side of the splice junction of said C-intein is an amino acid residue selected from the group consisting of cysteine, serine, and threonine.

163. A cell containing a first antigen recognition portion that binds a C3b-like receptor fused to the amino terminus of an N-intein of a split intein and a second antigen recognition portion fused to the carboxy terminus of a C-intein of said split intein, wherein the amino acid residue immediately at the C-terminal side of the splice junction of said C-intein is an amino acid residue selected from the group consisting of cysteine, serine, and threonine.

164. A method of treating a mammal having a disease or disorder or undesirable condition associated with the presence of a pathogen or pathogenic antigenic molecule, comprising administering to said mammal a therapeutically effective dose of a bispecific molecule comprising: (a) a first antigen recognition portion that binds a C3b-like receptor, said first antigen recognition portion comprising a monoclonal antibody; and (b) a second antigen recognition portion that binds an epitope of said pathogen or pathogenic antigenic molecule, said second antigen recognition portion being conjugated to a carboxy terminus of a heavy or light chain of said first antigen recognition portion.

165. A method of preventing a disease or disorder or undesirable condition associated with the presence of a pathogen or pathogenic antigenic molecule in a mammal, comprising administering to said mammal a therapeutically effective dose of a bispecific molecule comprising: (a) a first antigen recognition portion that binds a C3b-like receptor, said first antigen recognition portion comprising a monoclonal antibody; and (b) a second antigen recognition portion that binds an epitope of said pathogen or pathogenic antigenic molecule, said second antigen recognition portion being conjugated to a carboxy terminus of a heavy or light chain of said first antigen recognition portion.

166. The method of claim 164 or 165, wherein said second antigen recognition portion is conjugated to the C-terminus of a heavy chain of said first antigen recognition portion.

167. The method of claim 164 or 165, wherein said second antigen recognition portion is conjugated to the C-terminus of a light chain of said first antigen recognition portion.

168. The method of claim 164 or 165, wherein said second antigen recognition portion comprises two antigen recognition moieties each conjugated to the C-terminus of a different heavy chain of said first antigen recognition portion.

169. The method of claim 164 or 165, wherein said administering is intravenous.

170. The method of claim 164 or 165, wherein said mammal is a human, and said C3b-like receptor is CRI.

171. The method of claim 164 or 165, wherein said mammal is a non-human mammal.

172. The method of claim 170, wherein said pathogen is an autoimmune antigen.

173. The method of claim 170, wherein said pathogen is an infectious agent.

174. The method of claim 173, wherein said infectious agent is a virus.

175. The method of claim 174, wherein said virus is HIV-1 virus.

176. The method of claim 173, wherein said infectious agent is a bacterium.

177. The method of claim 176, wherein said bacterium is *Bacillus anthracis*.

178. The method of claim 173, wherein said infectious agent is a fungus.

179. The method of claim 173, wherein said infectious agent is a parasite.

180. The method of claim 179, wherein said parasite is a protozoan.

is a toxin.

182. A method of treating a mammal having an undesirable condition associated with the presence of a pathogen or pathogenic antigenic molecule comprising the steps of: (a) contacting a bispecific molecule with hematopoietic cells expressing a C3b-like receptor, to form a hematopoietic cell/bispecific molecule complex, wherein the bispecific molecule comprises (i) a first antigen recognition portion that binds said C3b-like receptor, said first antigen recognition portion comprising a monoclonal antibody; and (ii) a second antigen recognition portion that binds an epitope of said pathogen or pathogenic antigenic molecule, said second antigen recognition portion being conjugated to a carboxy terminus of a heavy or light chain of said first antigen recognition portion; and (b) administering said hematopoietic cell/bispecific molecule complex to said mammal in a therapeutically effective amount.

183. A method of treating a mammal having an undesirable condition associated with the presence of a pathogen or pathogenic antigenic molecule, comprising the step of administering a therapeutically effective amount of a hematopoietic cell/bispecific molecule complex to said mammal, said complex consisting essentially of a hematopoietic cell expressing a C3b-like receptor bound to one or more bispecific molecules, wherein said bispecific molecule comprises (a) a first antigen recognition portion that binds said C3b-like receptor, said first antigen recognition portion comprising a monoclonal antibody; and (b) a second antigen recognition portion that binds an epitope of said pathogen or pathogenic antigenic molecule, said second antigen recognition portion being conjugated to a carboxy terminus of a heavy or light chain of said first antigen recognition portion.

L5 ANSWER 25 OF 112 USPTAFULL on STN

2004:78909 Non-stochastic generation of genetic vaccines and enzymes.

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US 6713279 B1 20040330

APPLICATION: US 2000-498557 20000204 (9)

PRIORITY: US 1995-8311P 19951207 (60)

US 1995-8316P 19951207 (60)

DOCUMENT TYPE: Utility; GRANTED.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention provides methods of obtaining novel polynucleotides and encoded polypeptides by use of non-stochastic methods of directed evolution (DirectEvolution.TM.). These methods include non-stochastic polynucleotide site-saturation mutagenesis (Gene Site Saturation Mutagenesis.TM.) and non-stochastic polynucleotide reassembly (GeneReassembly.TM.). Through use of the claimed methods, genetic vaccines, enzymes, and other desirable molecules can be evolved towards desirable properties. For example, vaccine vectors can be obtained that exhibit increased efficacy for use as genetic vaccines. Vectors obtained by using the methods can have, for example, enhanced antigen expression, increased uptake into a cell, increased stability in a cell, ability to tailor an immune response, and the like. This invention provides methods of obtaining novel enzymes that have optimized physical &/or biological properties. Furthermore, this invention provides methods of obtaining a variety of novel biologically active molecules, in the fields of antibiotics, pharmacotherapeutics, and transgenic traits.

CLM What is claimed is:

1. A method of providing an immunomodulatory polynucleotide that has an optimized modulatory effect on an immune response, or encodes a polypeptide that has an optimized modulatory effect on an immune response, the method comprising creating a library of non-stochastically generated progeny polynucleotides from a parental polynucleotide set, thereby providing an immunomodulatory polynucleotide.

2. The method of claim 1 wherein the library of non-stochastically generated progeny polynucleotides is optimized by directed evolution of the parental polynucleotides, such that polypeptides encoded by the optimized progeny polynucleotides are enhanced in their modulatory effect on an immune response.

3. The method of claim 2, wherein said progeny polynucleotide whose modulatory effect on an immune response is optimized by directed evolution is introduced into a genetic vaccine vector.

4. The method of claim 2, wherein said method of directed evolution is selected from the group consisting of codon site-saturation mutagenesis, amino acid site-saturation mutagenesis, gene site saturation mutagenesis, introduction of mutations by non-stochastic polynucleotide reassembly methods, synthetic ligation polynucleotide reassembly, gene reassembly, **oligonucleotide**-directed saturation mutagenesis, in vivo

naturally occurring recombination processes which reduce sequence complexity, and any combination thereof.

5. The method of claim 4, wherein the method of directed evolution introduces at least at least one point mutation, addition, deletion, or chimerization, from one or more parental polynucleotides.

6. The method of claim 1, further comprising screening said library for progeny polynucleotides which encode polypeptides optimized for their immunomodulatory effect as compared to the parental polynucleotides.

7. The method of claim 1, wherein the optimized non-stochastically generated polynucleotide encodes a polypeptide that interacts with a cellular receptor.

8. The method of claim 7, wherein the cellular receptor is a macrophage scavenger receptor.

9. The method of claim 7, wherein the cellular receptor is selected from the group consisting of a cytokine receptor and a chemokine receptor.

10. The method of claim 9, wherein the chemokine receptor is CCR6.

11. The method of claim 7, wherein the polypeptide acts as an agonist or antagonist of the receptor.

12. The method of claim 1, wherein the library is screened by contacting replicable genetic packages, which express the encoded polypeptides of the optimized progeny polynucleotides as fusions with proteins displayed on the surface, with a plurality of cells that display the receptor.

13. The method of claim 12, further comprising identifying cells that exhibit a modulation of an immune response by the receptor.

14. The method of claim 12, wherein the replicable genetic package is selected from the group consisting of a bacteriophage, a cell, a spore, and a virus.

15. The method of claim 14, wherein the replicable genetic package is an M13 bacteriophage and the protein is encoded by geneIII or geneVIII.

16. The method of claim 1, further comprising introducing the optimized non-stochastically generated polynucleotide into a genetic vaccine vector and administering the vector to a subject.

17. The method of claim 16, wherein the peptide or polypeptide is an agonist or antagonist of the receptor.

18. The method of claim 1, wherein the optimized non-stochastically generated polynucleotide is inserted into an antigen-encoding nucleotide sequence of a genetic vaccine vector.

19. The method of claim 18, wherein the optimized non-stochastically generated polypeptide is introduced into a nucleotide sequence that encodes an HBsAg polypeptide.

20. The method of claim 1, wherein the optimized non-stochastically generated polynucleotide comprises a nucleotide sequence rich in unmethylated CpG.

21. The method of claim 1, wherein the optimized non-stochastically generated polynucleotide encodes a polypeptide that inhibits an allergic reaction.

22. The method of claim 21, wherein the polypeptide is selected from the group consisting of interferon- α , interferon- β , IL-10, IL-12, an antagonist of IL-4, an antagonist of IL-5, and an antagonist of IL-13.

23. The method of claim 1, wherein the optimized recombinant polynucleotide encodes an antagonist of IL-10.

24. The method of claim 23, wherein the antagonist of IL-10 is soluble or defective IL-10 receptor or IL-20/MDA-7.

25. The method of claim 1, wherein the optimized non-stochastically generated polynucleotide encodes a co-stimulator.

26. The method of claim 25, wherein the co-stimulator is B7-1 (CD80) or B7-2 (CD86).

27. The method of claim 26 wherein the screening step involves selecting

28. The method of claim 25, wherein the co-stimulator is CD1, CD40, CD154 (ligand for CD40) or CD150 (SLAM).
29. The method of claim 25, wherein the co-stimulator is a cytokine.
30. The method of claim 29, wherein the cytokine is selected from the group consisting of IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, GM-CSF, G-CSF, TNF- α , IFN- α , IFN- γ , and IL-20 (MDA-7).
31. The method of 30, wherein the library of non-stochastically generated polynucleotides is screened by testing the ability of cytokines encoded by the non-stochastically generated polynucleotides to activate cells which contain a receptor for the cytokine.
32. The method of claim 31, wherein the cells contain a heterologous nucleic acid that encodes the receptor for the cytokine.
33. The method of 30, wherein the cytokine is interleukin-12 and screening is performed by growing mammalian cells which contain the genetic vaccine vector in a culture medium and detecting whether T cell proliferation or T cell differentiation is induced by contact with the culture medium.
34. The method of 30, wherein the cytokine is interferon- γ .
35. The method of claim 34, wherein the library is screened by contacting replicable genetic packages, which express the encoded polypeptides of the optimized progeny polynucleotides as fusions with proteins displayed on the surface, with a plurality of B cells that display the receptor.
36. The method of claim 35, further comprising identifying phage library members that are capable of inhibiting proliferation of the B cells.
37. The method of claim 30, wherein the immune response of interest is differentiation of T cells to T_{H1} cells.
38. The method of claim 37, wherein said immune response of interest is screened by contacting a population of T cells with the cytokines encoded by the members of the library of recombinant polynucleotides and identifying library members that encode a cytokine that induces the T cells to produce IL-2 and interferon- γ .
39. The method of claim 29, wherein the cytokine encoded by the optimized non-stochastically generated polynucleotide exhibits reduced immunogenicity compared to a cytokine encoded by a non-optimized polynucleotide.
40. The method of claim 39, wherein the reduced immunogenicity is detected by introducing a cytokine encoded by the non-stochastically generated polynucleotide into a subject and determining whether an immune response is induced against the cytokine.
41. The method of claim 31, wherein the cell is tested for ability to costimulate an immune response.
42. The method of claim 1, wherein the optimized recombinant polynucleotide encodes a cytokine antagonist.
43. The method of claim 42, wherein the cytokine antagonist is selected from the group consisting of a soluble cytokine receptor, a transmembrane cytokine receptor having a defective signal sequence, IL-10R and IL-4R.
44. The method of claim 1, wherein the optimized non-stochastically generated polynucleotide encodes a polypeptide capable of inducing a predominantly T_{H1} immune response.
45. The method of claim 1, wherein the optimized non-stochastically generated polynucleotide encodes a polypeptide capable of inducing a predominantly T_{H2} immune response.
46. The method of claim 1, wherein said optimized modulatory effect on an immune response is a decrease in an unwanted modulatory effect on an immune response.
47. The method of claim 46, wherein said method generates a molecule having a decreased ability to elicit an immune response from a host recipient of said molecule.

48. The method of claim 47, wherein said recipient can be a human or an animal host.

49. The method of claim 48, wherein said method generates a molecule having decreased antigenicity with respect to at least one host recipient of said molecule.

50. The method of claim 49, wherein said recipient can be a human or an animal host.

51. The method of claim 1, wherein said optimized modulatory effect on an immune response is both a decrease in a first unwanted modulatory effect on an immune response and an increase in a second desirable modulatory effect on an immune response.

52. The method of claim 51, wherein the first and the second recipient hosts can be the same or different.

53. The method of claim 51, wherein each of the first and the second recipient hosts can be human or animal.

54. The method of claim 51, wherein said method generates a molecule having both a decreased ability to elicit a first immune response from a first host recipient of said molecule and an increased ability to elicit a second immune response from a second host recipient of said molecule.

55. The method of claim 54, wherein the first and the second recipient hosts can be the same or different.

56. The method of claim 54, wherein each of the first and the second recipient hosts can be a human or animal.

57. The method of claim 51, wherein said method generates a molecule having both a first decreased antigenicity with respect to at least one host recipient of said molecule and a second decreased antigenicity with respect to at least one host recipient of said molecule.

58. The method of claim 46, wherein said first and said second modulatory effect on an immune response are evolved for respectively a first and a second module on the same multimodule vaccine vector.

59. The method of claim 58, wherein said module is selected from the group of modules consisting of an antigen coding sequence, a polyadenylation sequence, a sequence coding for a co-stimulatory molecule, a sequence coding for an inducible repressor or transactivator, a eukaryotic origin of replication, a prokaryotic origin of replication, a sequence coding for a prokaryotic marker, an enhancer, a promoter, an operator, an intron, or derivative fragments or analogs thereof, and any combination thereof.

60. The method of claim 1, wherein the optimized modulatory effect on an immune response is comprised of an increase in the stability of the immunomodulatory (IM) polynucleotide or polypeptide encoded thereby.

61. The method of claim 60, wherein said method generates a molecule having an increased stability ex vivo.

62. The method of claim 60 wherein said method generates a molecule having increased stability in vivo, with respect to any means of biological elimination or degradation, upon administration to a host recipient.

63. The method of claim 1, wherein the immunomodulatory (IM) polynucleotide or polypeptide encoded thereby has an optimized modulatory effect on an immune response in an animal or human host recipient.

64. The method of claim 63, wherein said method generates an optimized genetic vaccine for any human and/or non-human recipients.

65. A method of providing an optimized non-stochastically generated polynucleotide that has a modulatory effect on an immune response said method comprising non-stochastically reassembling at least two parental template polynucleotides, each of which encodes a molecule that is involved in modulating an immune response, thereby providing a library of non-stochastically generated polynucleotides.

66. The method of claim 65, wherein the first and second parental templates differ from each other in two or more nucleotides.

67. The method of claim 65, further comprising screening the library to identify at least one optimized non-stochastically generated

ability to modulate an immune response in comparison to a parental polynucleotide from which the library was created.

68. The method of claim 65, wherein an optimized non-stochastically generated polynucleotide is subjected to at least one further round of non-stochastic reassembly with at least one additional polynucleotide to produce additional working libraries of recombinant polynucleotides.

69. The method of claim 68, wherein said additional working libraries are screened to identify at least one further optimized non-stochastically generated polynucleotide which encodes a polypeptide that has been optimized for its immunomodulatory effect when compared to the parental polynucleotide from which the library was created.

70. A method of providing an optimized polynucleotide that encodes an accessory molecule that improves the transport or presentation of antigens by a cell, said method comprising creating a library of non-stochastically generated polynucleotides by subjecting to optimization by non-stochastic directed evolution a parental polynucleotide set in which is encoded all or part of the accessory molecule.

71. The method of claim 70, further comprising screening the library to identify an optimized non-stochastically generated progeny polynucleotide that encodes a recombinant molecule that confers upon a cell an increased or decreased ability to transport or present an antigen on a surface of the cell as compared to an accessory molecule encoded by template polynucleotides not subjected to the non-stochastic reassembly.

72. The method of claim 70, wherein said method of directed evolution is selected from the group consisting of codon site-saturation mutagenesis, amino acid site-saturation mutagenesis, gene site saturation mutagenesis, introduction of mutations by non-stochastic polynucleotide reassembly methods, synthetic ligation polynucleotide reassembly, gene reassembly, **oligonucleotide**-directed saturation mutagenesis, in vivo reassortment of polynucleotide sequences having partial homology, naturally occurring recombination processes which reduce sequence complexity, and any combination thereof.

73. The method of claim 70, wherein said method generates an optimized molecule for any human and/or non-human recipients.

74. The method of claim 70, further comprising forming a library of vectors by introducing the library of non-stochastically generated polynucleotides into a genetic vaccine vector that encodes an antigen.

75. The method of claim 74, wherein the library of vectors is introduced into mammalian cells.

76. The method of claim 75, wherein said cells that exhibit increased or decreased immunogenicity to the antigen are identified.

77. The method of claim 70, wherein the accessory molecule comprises a proteasome or a TAP polypeptide.

78. The method of claim 70, wherein the accessory molecule comprises a cytotoxic T-cell inducing sequence.

79. The method of claim 78, wherein the cytotoxic T-cell inducing sequence is obtained from a hepatitis B surface antigen.

80. The method of claim 70, wherein the accessory molecule comprises an immunogenic agonist sequence.

81. A method of providing an immunomodulatory polynucleotide that has an optimized expression in a recombinant expression host, said method comprising creating a library of non-stochastically generated progeny polynucleotides from a parental polynucleotide set, thereby providing an immunomodulatory polynucleotide.

82. The method of claim 81, wherein the library of non-stochastically generated polynucleotides is optimized by directed evolution.

83. The method of claim 82, wherein said method of directed evolution is selected from the group consisting of codon site-saturation mutagenesis, amino acid site-saturation mutagenesis, gene site saturation mutagenesis, introduction of mutations by non-stochastic polynucleotide reassembly methods, synthetic ligation polynucleotide reassembly, gene reassembly, **oligonucleotide**-directed saturation mutagenesis, in vivo reassortment of polynucleotide sequences having partial homology, naturally occurring recombination processes which reduce sequence

84. The method of claim 82, further comprising screening a library of non-stochastically generated progeny polynucleotides to identify an optimized non-stochastically generated progeny polynucleotide that has an optimized expression in a recombinant expression host as compared to the expression of parental polynucleotides.

85. The method of claim 82, wherein the recombinant expression host is a prokaryote.

86. The method of claim 82, wherein the recombinant expression host is a eukaryote.

87. The method of claim 86, wherein the recombinant expression host is a plant.

88. The method of claim 87, wherein the recombinant expression host is a monocot or dicot.

89. A method of producing a progeny polynucleotide set by subjecting a double-stranded circular parental polynucleotide molecule to mutagenesis, said method comprising synthesizing by means of a polymerase-catalyzed amplification reaction a first progeny polynucleotide strand comprised of said first **primer** and a second progeny polynucleotide strand comprised of said second **primer**, wherein the first progeny polynucleotide strand and the second progeny polynucleotide strand form a double-stranded mutagenized circular polynucleotide product, and wherein at least one of said **primers** contains a non-stochastic mutagenic cassette with respect to the parental polynucleotide molecule, thereby producing a progeny polynucleotide set.

90. The method of claim 89, wherein said non-stochastic mutagenic cassette contained in said at least one **primer** is degenerate in nature.

91. The method of claim 90, wherein a degenerate progeny polynucleotide set is produced.

92. A method of producing a set of progeny polypeptides, in which a non-stochastic range of single amino acid substitutions is represented at each amino acid position, from a template polypeptide set, said method comprising subjecting a codon-containing template polynucleotide to polymerase-based amplification using a degenerate **oligonucleotide** for each codon to be mutagenized.

93. The method of claim 92, wherein said method generates from at least one to twenty different amino acids at each amino acid site along a parental polypeptide template.

94. The method of claim 92, wherein said degenerate **oligonucleotides** is comprised of a first homologous sequence and a degenerate trinucleotide cassette.

95. The method of claim 92, wherein said degenerate **oligonucleotide** is comprised of a first homologous sequence, a degenerate trinucleotide cassette, and a second homologous sequence.

96. The method of claim 92, wherein said degenerate trinucleotide cassette is comprised of a first mononucleotide cassette selected from the group consisting of: a degenerate A/C mononucleotide cassette, a degenerate A/G mononucleotide cassette, a degenerate A/T mononucleotide cassette, a degenerate C/G mononucleotide cassette, a degenerate C/T mononucleotide cassette, a degenerate G/T mononucleotide cassette, a degenerate C/G/T mononucleotide cassette, a degenerate A/G/T mononucleotide cassette, a degenerate A/C/T mononucleotide cassette, a degenerate A/C/G mononucleotide cassette, and a degenerate A/C/G/T mononucleotide cassette.

97. The method of claim 96, wherein said degenerate trinucleotide cassette is further comprises a second and a third mononucleotide cassette, each selected from the group consisting of: a degenerate A/C mononucleotide cassette, a degenerate A/G mononucleotide cassette, a degenerate A/T mononucleotide cassette, a degenerate C/G mononucleotide cassette, a degenerate C/T mononucleotide cassette, a degenerate G/T mononucleotide cassette, a degenerate C/G/T mononucleotide cassette, a degenerate A/G/T mononucleotide cassette, a degenerate A/C/T mononucleotide cassette, a degenerate A/C/G mononucleotide cassette, a degenerate A/C/G/T mononucleotide cassette, a non-degenerate A mononucleotide cassette, and a non-degenerate C mononucleotide cassette, a non-degenerate G mononucleotide cassette, and a non-degenerate T mononucleotide cassette.

is selected from the group consisting of: a degenerate N,N,N trinucleotide cassette, a degenerate N,N,G/T trinucleotide cassette, a degenerate N,N,G/C trinucleotide cassette, a degenerate N,N,A/C/G trinucleotide cassette, a degenerate N,N,A/G/T trinucleotide cassette, and a degenerate N,N,C/G/T trinucleotide cassette.

99. The method of claim 92, wherein said degenerate **oligonucleotide** is comprised of a first homologous sequence and a plurality of trinucleotide cassettes.

100. The method of claim 99, wherein said method generates a progeny polypeptide having a plurality of concurrent single amino acid changes, at each amino acid site, with respect to a parental polypeptide template.

101. The method of claim 9, wherein each of said degenerate trinucleotide cassettes is comprised of a first mononucleotide cassette selected from the group consisting of: a degenerate A/C mononucleotide cassette, a degenerate A/G mononucleotide cassette, a degenerate A/T mononucleotide cassette, a degenerate C/G mononucleotide cassette, a degenerate C/T mononucleotide cassette, a degenerate G/T mononucleotide cassette, a degenerate C/G/T mononucleotide cassette, a degenerate A/G/T mononucleotide cassette, a degenerate A/C/T mononucleotide cassette, a degenerate A/C/G mononucleotide cassette, and a degenerate A/C/G/T mononucleotide cassette.

102. The method of claim 101, wherein each of said degenerate trinucleotide cassettes further comprises a second and third mononucleotide cassette, each selected from the group consisting of: a degenerate A/C mononucleotide cassette, a degenerate A/G mononucleotide cassette, a degenerate A/T mononucleotide cassette, a degenerate C/G mononucleotide cassette, a degenerate C/T mononucleotide cassette, a degenerate G/T mononucleotide cassette, a degenerate C/G/T mononucleotide cassette, a degenerate A/G/T mononucleotide cassette, a degenerate A/C/T mononucleotide cassette, a degenerate A/C/G mononucleotide cassette, a degenerate A/C/G/T mononucleotide cassette, a non-degenerate A mononucleotide cassette, a non-degenerate C mononucleotide cassette, a non-degenerate G mononucleotide cassette, and a non-degenerate T mononucleotide cassette.

103. The method of claim 99, where said degenerate trinucleotide cassette is selected from the group consisting of: a degenerate N,N,N trinucleotide cassette, a degenerate N,N,G/T trinucleotide cassette, a degenerate N,N,G/C trinucleotide cassette, a degenerate N,N,A/C/G trinucleotide cassette, a degenerate N,N,A/G/T trinucleotide cassette, and a degenerate N,N,C/G/T trinucleotide cassette.

104. The method of claim 92, wherein said degenerate **oligonucleotide** is comprised of a first homologous sequence, and a plurality of trinucleotide cassettes, and a second homologous sequence.

105. The method of claim 92, further comprising screening the progeny polypeptides to identify those that display a desirable change with respect to at least one molecular property as compared with its parental polypeptide.

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FILE 'USPATFULL' ENTERED AT 22:55:09 ON 03 FEB 2006
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L1 12 S E3
L2 1163 S (DENGUE VIRUS)
L3 727 S L2 AND (PCR OR POLYMERASE CHAIN REACTION)
L4 178 S L3 AND (PRIMER?/CLM OR OLIGONUCLEOTIDE?/CLM)
L5 112 S L4 AND AY<2003

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L5 ANSWER 26 OF 112 USPATFULL on STN

2004:51756 Chimeric GB virus B (GBV-B).

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Board of Regents, The University of Texas System and Institut Pasteur

(non-U.S. corporation)

US 2004039187 A1 20040226

APPLICATION: US 2002-189359 A1 20020703 (10)

AB The present invention relates generally to the fields of biochemistry, molecular biology, and virology. More particularly, it relates to the identification of GB virus B (GBV-B)/HCV chimeras. The invention involves nucleic acid constructs and compositions encoding GBV-B/HCV chimera, including at least part of a 5' NTR derived from a HCV 5' NTR. This construct, and chimeric versions of it, may be employed to study GBV-B and related hepatitis family members, such as hepatitis C virus. The invention thus includes methods of preparing GBV-B/HCV chimeric sequences, constructs, and viruses, as well as methods of employing these compositions.

CLM What is claimed is:

1. An isolated polynucleotide encoding a 3' sequence of the GBV-B genome.
2. The polynucleotide of claim 1, wherein said polynucleotide has the sequence of SEQ ID NO:1.
3. The polynucleotide of claim 1, wherein said polynucleotide is DNA.
4. The polynucleotide of claim 1, wherein said polynucleotide is RNA.
5. A viral expression construct comprising a polynucleotide encoding a 3' sequence of the GBV-B genome.
6. The expression construct of claim 5, wherein said polynucleotide has 50 contiguous nucleotides of SEQ ID NO:1.
7. The expression construct of claim 5, wherein said polynucleotide has 100 contiguous nucleotides of SEQ ID NO:1.
8. The expression construct of claim 5, wherein said polynucleotide has 150 contiguous nucleotides of SEQ ID NO:1.
9. The expression construct of claim 5, wherein said polynucleotide has the sequence of SEQ ID NO:1.
10. The expression construct of claim 5, wherein said polynucleotide comprises at least 250 contiguous nucleotides of SEQ ID NO:2.
11. The expression construct of claim 5, wherein said polynucleotide comprises at least 500 contiguous nucleotides of SEQ ID NO:2.
12. The expression construct of claim 5, wherein said polynucleotide comprises at least 1000 contiguous nucleotides of SEQ ID NO:2.
13. The expression construct of claim 5, wherein said polynucleotide comprises at least 5000 contiguous nucleotides of SEQ ID NO:2.
14. The expression construct of claim 5, wherein said polynucleotide comprises SEQ ID NO:2.
15. The expression construct of claim 5, wherein said construct is a plasmid.
16. The expression construct of claim 5, wherein said construct is a virus.
17. The expression construct of claim 5, further defined as a construct for the expression of GBV-B.
18. The expression construct of claim 5, further defined as a construct for the expression of a chimeric GBV-B/HCV virus.
19. A method of producing a virus comprising: introducing into a host cell a viral expression construct comprising a polynucleotide encoding a 3' sequence of GBV-B; and culturing said host cell under conditions permitting production of a virus from said construct.
20. The method of claim 19, wherein said polynucleotide comprises 100 contiguous nucleotides from SEQ ID NO:1.
21. The method of claim 20, wherein said polynucleotide comprises SEQ ID NO:1.
22. The method of claim 19, wherein said polynucleotide comprises at least 250 contiguous nucleotides of SEQ ID NO:2.
23. The method of claim 19, wherein said polynucleotide comprises at least 500 contiguous nucleotides of SEQ ID NO:2.

least 1000 contiguous nucleotides of SEQ ID NO:2.

25. The method of claim 19, wherein said polynucleotide comprises at least 5000 contiguous nucleotides of SEQ ID NO:2.

26. The method of claim 19, wherein said polynucleotide comprises SEQ ID NO:2.

27. The method of claim 19, wherein said host cell is a prokaryotic cell.

28. The method of claim 19, wherein said host cell is a eukaryotic cell.

29. The method of claim 28, wherein said host cell is in an animal.

30. The method of claim 19, wherein said polynucleotide comprises synthetic RNA.

31. The method of claim 19, wherein said polynucleotide comprises synthetic DNA.

32. The method of claim 19, further comprising the step of isolating virus from said host cell.

33. The method of claim 32, wherein said virus is purified to homogeneity.

34. An **oligonucleotide** between about 10 and about 259 consecutive bases of SEQ ID NO:1.

35. The **oligonucleotide** of claim 34, wherein said **oligonucleotide** is about 15 bases in length.

36. The **oligonucleotide** of claim 34, wherein said **oligonucleotide** is about 20 bases in length.

37. The **oligonucleotide** of claim 34, wherein said **oligonucleotide** is about 25 bases in length.

38. The **oligonucleotide** of claim 34, wherein said **oligonucleotide** is about 30 bases in length.

39. The **oligonucleotide** of claim 34, wherein said **oligonucleotide** is about 35 bases in length.

40. The **oligonucleotide** of claim 34, wherein said **oligonucleotide** is about 50 bases in length.

41. The **oligonucleotide** of claim 34, wherein said **oligonucleotide** is about 100 bases in length.

42. The **oligonucleotide** of claim 34, wherein said **oligonucleotide** is about 150 bases in length.

43. The **oligonucleotide** of claim 34, wherein said **oligonucleotide** is about 200 bases in length.

44. The **oligonucleotide** of claim 34, wherein said **oligonucleotide** is about 259 bases in length.

45. A method for identifying a compound active against a viral infection comprising: providing a virus expressed from a viral construct comprising a 3' sequence of a GBV-B virus; contacting said virus with a candidate substance; and comparing the infectious ability of the virus in the presence of said candidate substance with the infectious ability of the virus in a similar system in the absence of said candidate substance.

46. The method of claim 45, wherein the virus is a GBV-B virus.

47. The method of claim 45, wherein the virus is a GBV-B/HCV chimera.

48. A compound active against a viral infection identified according to a method comprising: providing a virus expressed from a viral construct comprising a 3' sequence of a GBV-B virus; contacting said virus with a candidate substance; and comparing the infectious ability of the virus in the presence of said candidate substance with the infectious ability of the virus in a similar system in the absence of said candidate substance.

49. The compound of claim 48, wherein the virus is a GBV-B virus.

51. The isolated polynucleotide of claim 1, further comprising at least part of a 5' NTR sequence derived from a HCV 5' NTR.
52. The isolated polynucleotide of claim 51, wherein domain III of the 5' NTR is derived from a HCV 5'NTR.
53. The isolated polynucleotide of claim 51, wherein domain Ib of GBV-B is deleted.
54. The isolated polynucleotide of claim 51, further comprising at least part of a structural protein coding region of HCV.
55. The isolated polynucleotide of claim 51, further comprising at least part of a non-structural protein coding region of HCV.
56. The method of claim 47, further comprising at least part of a 5' NTR sequence derived from a HCV 5' NTR.
57. The method of claim 56, wherein domain III of the 5' NTR is derived from a HCV 5'NTR.
58. The method of claim 56, wherein domain Ib of GBV-B is deleted.
59. The compound of claim 50, further comprising at least part of a 5' NTR sequence derived from a HCV 5' NTR.
60. The compound of claim 59, wherein domain III of the 5' NTR is derived from a HCV 5' NTR.
61. The compound of claim 59, wherein domain Ib of GBV-B is deleted.
62. An isolated polynucleotide comprising a chimeric GBV-B genome, wherein at least part, but not all of a 5' NTR sequence is derived from a HCV 5' NTR.
63. The polynucleotide of claim 62, wherein at least domain I, II, III, or IV of the 5' NTR is derived from a HCV 5' NTR, but not all.
64. The polynucleotide of claim 62, wherein domain I of the 5' NTR is derived from a HCV 5'NTR.
65. The polynucleotide of claim 62, wherein domain II of the 5' NTR is derived from a HCV 5'NTR.
66. The polynucleotide of claim 62, wherein domain III of the 5' NTR is derived from a HCV5'NTR.
67. The polynucleotide of claim 66, wherein 5' NTR domain Ib of GBV-B is deleted.
68. The polynucleotide of claim 62, wherein domain IV of the 5' NTR is derived from a HCV 5'NTR.
69. The polynucleotide of claim 62, wherein domain I and domain II of the 5' NTR is derived from a HCV 5'NTR.
70. The polynucleotide of claim 62, wherein domain I and domain III of the 5' NTR is derived from a HCV 5'NTR.
71. The polynucleotide of claim 62, wherein domain I and domain IV of the 5' NTR is derived from a HCV 5'NTR.
72. The polynucleotide of claim 62, wherein domain II and domain III of the 5' NTR is derived from a HCV 5'NTR.
73. The polynucleotide of claim 62, wherein domain II and domain IV of the 5' NTR is derived from a HCV 5'NTR.
74. The polynucleotide of claim 62, wherein domain III and domain IV of the 5' NTR is derived from a HCV 5'NTR.
75. The polynucleotide of claim 62, wherein domain II, domain III and domain IV of the 5' NTR is derived from a HCV 5'NTR.
76. The polynucleotide of claim 75, wherein 5' NTR domain Ib of GBV-B is deleted.
77. The polynucleotide of claim 62, wherein said polynucleotide is DNA.
78. The polynucleotide of claim 62, wherein said polynucleotide is RNA.

polynucleotide, wherein at least a part of the 5' NTR sequence is derived from a HCV 5' NTR.

80. The viral expression construct of claim 79, wherein domain III of the 5' NTR is derived from a HCV 5' NTR.

81. The viral expression construct of claim 79, further comprising a deletion of the GBV-B 5' NTR domain Ib region.

82. The viral expression construct of claim 79, wherein said construct is a plasmid.

83. The viral expression construct of claim 79, wherein said construct is a virus.

84. The viral expression construct of claim 79, further defined as a construct for the expression of a chimeric GBV-B/HCV virus.

85. A hepatotropic virus comprising a chimeric GBV-B polynucleotide, wherein at least a part of the 5' NTR sequence is derived from a HCV 5' NTR.

86. The hepatotropic virus of claim 85, wherein domain III of the 5' NTR is derived from a HCV 5' NTR.

87. The hepatotropic virus of claim 85, further comprising a deletion of the GBV-B 5' NTR domain Ib region.

88. The hepatotropic virus of claim 86, wherein the virus propagates in vivo.

89. The hepatotropic virus of claim 85, further defined as a construct for the expression of a chimeric GBV-B/HCV virus.

90. A method of producing a virus comprising: introducing into a host cell a viral expression construct comprising a chimeric GBV-B polynucleotide encoding at least part of a 5' NTR sequence derived from a HCV 5' NTR sequence; and culturing said host cell under conditions permitting production of a virus from said construct.

91. The method of claim 90, wherein said polynucleotide comprises at least a 5' NTR domain I derived from a HCV 5' NTR.

92. The method of claim 90, wherein said polynucleotide comprises at least a 5' NTR domain II derived from a HCV 5' NTR.

93. The method of claim 90, wherein said polynucleotide comprises at least a 5' NTR domain III derived from a HCV 5' NTR.

94. The method of claim 93, wherein said polynucleotide comprises a deletion of 5' NTR domain Ib of GBV-B.

95. The method of claim 90, wherein said polynucleotide comprises at least a 5' NTR domain I and domain II derived from a HCV 5' NTR.

96. The method of claim 90, wherein said polynucleotide comprises at least a 5' NTR domain I and domain IV derived from a HCV 5' NTR.

97. The method of claim 90, wherein said polynucleotide comprises at least a 5' NTR domain I, domain II and domain III derived from a HCV 5' NTR.

98. The method construct of claim 90, wherein said polynucleotide comprises a 5' NTR derived from a HCV 5' NTR.

99. The method of claim 90, wherein said polynucleotide comprises at least a 5' NTR domain II and domain III derived from a HCV 5' NTR.

100. The method of claim 90, wherein said polynucleotide comprises at least a 5' NTR domain III and domain IV derived from a HCV 5' NTR.

101. The method of claim 90, wherein said host cell is a eukaryotic cell.

102. The method of claim 101, wherein said host cell is in an animal.

103. The method of claim 90, wherein said polynucleotide comprises synthetic RNA.

104. The method of claim 90, further comprising the step of isolating virus from said host cell.

homogeneity.

106. A method for identifying a compound active against a viral infection comprising: providing a virus expressed from a viral construct comprising at least part of a 5' NTR derived from a HCV 5' NTR; contacting said virus with a candidate substance; and comparing the infectious ability of the virus in the presence of said candidate substance with the infectious ability of the virus in a similar system in the absence of said candidate substance.

107. The method of claim 106, wherein said polynucleotide comprises at least a 5' NTR domain III derived from a HCV 5' NTR.

108. The method of claim 106, wherein said polynucleotide comprises a deletion of 5' NTR domain Ib of GBV-B.

109. A compound active against a viral infection identified according to a method comprising: providing a virus expressed from a viral construct comprising at least part of a 5' NTR derived from a HCV 5' NTR; contacting said virus with a candidate substance; and comparing the infectious ability of the virus in the presence of said candidate substance with the infectious ability of the virus in a similar system in the absence of said candidate substance.

110. The method of claim 109, wherein said polynucleotide comprises at least a 5' NTR domain III derived from a HCV 5' NTR.

111. The method of claim 109, wherein said polynucleotide comprises a deletion of 5' NTR domain Ib of GBV-B.

L5 ANSWER 27 OF 112 USPTAFULL on STN

2004:31070 Kits for a selective assay for determining the identity of live microorganisms in a mixed culture.

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Uren, Jack R., Kirkland, WA, UNITED STATES

Saigene Corporation, Seattle, WA (U.S. corporation)

US 2004023208 A1 20040205

APPLICATION: US 2002-285819 A1 20021031 (10)

PRIORITY: US 1998-79684P 19980327 (60)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention provides methods for determining the identity of an unknown live microorganism in a mixed culture. The microorganism can be a bacterium, fungus, virus, or protozoan. The invention further provides an assay for determining the ability of a selected microorganism in a mixed culture to replicate in the presence of a chemical agent. Kits for determining the identity of a live microorganism in a mixed culture and for determining the ability of a microorganism in a mixed culture to replicate are also provided.

CLM What is claimed is:

1. An assay for determining the identity of an unknown live microorganism in a mixed culture, said assay comprising the steps of: i. culturing the microorganism in an aqueous medium, said medium able to culture one or more microorganisms other than the unknown microorganism, and comprising a nucleic acid analog which is recognized by an analog-specific binding member and which is incorporated into nucleic acids of replicating cells or virions of the unknown microorganism; ii. lysing the microorganisms to release nucleic acids of the unknown microorganism, and other microorganisms which may be present; iii. capturing nucleic acids incorporating the analog using the analog-specific binding member; iv. separating the captured nucleic acids from any nucleic acids which have not been captured; v. amplifying captured nucleic acids originating from the unknown microorganism; and, vi. determining the identity of the microorganism from which the captured and amplified nucleic acids originate.

2. An assay of claim 1 wherein step (v) is a selective amplification of a subset of the captured nucleic acids.

3. An assay of claim 1 wherein the identity of the microorganism in step (vi) is determined by hybridization of the captured and amplified nucleic acids to a species-specific nucleic acid sequence.

4. An assay of claim 1 wherein the unknown microorganism is a bacterium.

5. An assay of claim 1 wherein the unknown microorganism is a pathogen.

6. An assay of claim 5 wherein the pathogen is a pathogen of a mammal.

7. An assay of claim 1 wherein the pathogen is a virus.

9. An assay of claim 1 wherein the pathogen is a protozoan.
10. An assay of claim 1 wherein the mixed culture is of two or more strains of the same species of microorganism.
11. An assay of claim 1 wherein the analog is bromodeoxyuridine.
12. An assay of claim 11 wherein the specific binding member is an antibody.
13. An assay of claim 1 wherein the aqueous medium further comprises an antibiotic.
14. An assay for determining the ability of a selected microorganism in a mixed culture to replicate in the presence of a chemical agent comprising the steps of: i. culturing the selected microorganism in an aqueous medium or cell culture, said medium or cell culture able to culture one or more microorganisms other than the selected microorganism, comprising a nucleic acid analog recognized by an analog-specific binding member and which is incorporated into nucleic acids of replicating cells or virions of the selected microorganism; ii. lysing the microorganisms to release nucleic acids of the selected microorganism, and other microorganisms which may be present; iii. capturing nucleic acids incorporating the analog using the analog-specific binding member; iv. separating the captured nucleic acids from any nucleic acids which have not been captured; v. amplifying captured nucleic acids originating from the selected microorganism; and, vi. detecting the presence or absence of captured nucleic acids, wherein the series of steps i-vi is conducted at least twice, at least once with the series of steps inclusive of a chemical agent added to the culture media and at least once with the series of steps not having a chemical agent added to the culture media.
15. An assay of claim 14 wherein the amplification of step v is by **polymerase chain reaction**.
16. An assay of claim 14 wherein the analog binding member is attached to a solid support.
17. A kit for determining the identity of a microorganism in a mixed culture comprising: i. a DNA analog which is recognized by an analog-specific binding member and which is permissive of incorporation into nucleic acids of the microorganism; ii. an analog-specific binding member for capturing nucleic acids incorporating the DNA analog; iii. **primers** for amplifying nucleic acids from a microorganism whose identity is of interest, and, iv. instructions for using components i, ii, and iii in an assay for determining the identity of the microorganism.
18. A kit of claim 17 wherein the **primers** are specific for amplifying microorganisms of a species or strain of interest.
19. A kit of claim 17, further comprising species-specific nucleic acid sequences.
20. A kit of claim 17 wherein the DNA analog is bromodeoxyuridine.
21. A kit of claim 17 wherein the analog specific binding member is an antibody.
22. A kit of claim 17 which further comprises an antibiotic.
23. A kit for determining the ability of a microorganism in a mixed culture to replicate comprising: i. a DNA analog which is recognized by an analog-specific binding member and which is permissive of incorporation into nucleic acids of the microorganism; ii. an analog-specific binding member for capturing nucleic acids incorporating the DNA analog; iii. **primers** for amplifying nucleic acids from the microorganism whose ability to replicate is of interest, and, iv. instructions for using components i, ii, and iii in an assay for determining the ability of the microorganism to replicate.
24. A kit of claim 23 wherein the **primers** are specific for amplifying microorganisms of a species or strain of interest.
25. A kit of claim 23, further comprising species-specific nucleic acid sequences.
26. A kit of claim 23 wherein the DNA analog is bromodeoxyuridine.
27. A kit of claim 23 wherein the analog specific binding member is an

28. A kit of claim 23 which further comprises an antibiotic.

L5 ANSWER 28 OF 112 USPATFULL on STN

2003:326950 Polynucleotide encoding a polypeptide having heparanase activity and expression of same in genetically modified cells.

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Vlodavsky, Israel, Mevasseret Zion, ISRAEL

Feinstein, Elena, Rehovot, ISRAEL

Insight Strategy & Marketing Ltd., Rehovot, ISRAEL (non-U.S.

corporation)Hadasit Medical Research Services and Development Ltd., Jerusalem, ISRAEL (non-U.S. corporation)

US 6664105 B1 20031216

APPLICATION: US 1999-435739 19991108 (9)

DOCUMENT TYPE: Utility; GRANTED.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A polynucleotide (hpa) encoding a polypeptide having heparanase activity, vectors including same, genetically modified cells expressing heparanase, a recombinant protein having heparanase activity and antisense oligonucleotides and constructs for modulating heparanase expression are provided.

CLM What is claimed is:

1. An antisense **oligonucleotide** comprising a polynucleotide of a least 10 bases which specifically targets and inhibits the expression of the polynucleotide of SEQ ID No: 9 or SEQ ID NO: 13, wherein the polynucleotides SEQ ID NO: 9 and 13 encode polypeptides having heparanase catalytic activity.

2. The antisense **oligonucleotide** of claim 1, wherein said polypeptide having heparanase catalytic activity is as set forth in SEQ ID NOS: 10 and 14.

3. An antisense nucleic acid construct comprising a promoter sequence operably-linked to a polynucleotide sequence encoding the antisense **oligonucleotide** of claim 1.

4. The antisense nucleic acid construct of claim 3, wherein said polypeptide having heparanase catalytic activity is as set forth in SEQ ID NOS: 10, or 14.

5. A nucleic acid construct comprising SEQ ID NO: 13, encoding a polypeptide having heparanase catalytic activity.

L5 ANSWER 29 OF 112 USPATFULL on STN

2003:319777 Secondary structure defining database and methods for determining identity and geographic origin of an unknown bioagent for environmental testing thereby.

Ecker, David J., Encinitas, CA, UNITED STATES

Griffey, Richard, Vista, CA, UNITED STATES

Sampath, Rangarajan, San Diego, CA, UNITED STATES

Hofstadler, Steven, Oceanside, CA, UNITED STATES

McNeil, John, La Jolla, CA, UNITED STATES

Crooke, Stanley T., Carlsbad, CA, UNITED STATES

US 2003225529 A1 20031204

APPLICATION: US 2002-323210 A1 20021218 (10)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates generally to the field of investigational bioinformatics and more particularly to secondary structure defining databases. The present invention further relates to methods for interrogating a database as a source of molecular masses of known bioagents for comparing against the molecular mass of an unknown or selected bioagent to determine either the identity of the selected bioagent, and/or to determine the origin of the selected bioagent. The identification of the bioagent is important for determining a proper course of treatment and/or irradiation of the bioagent in such cases as biological warfare. Furthermore, the determination of the geographic origin of a selected bioagent will facilitate the identification of potential criminal identity.

CLM What is claimed is:

1. A method of identifying an unknown bioagent in an environmental sample using a database of molecular masses of known bioagents comprising: contacting nucleic acid from said bioagent in said environmental sample with at least one pair of **oligonucleotide primers** that hybridize to sequences of said nucleic acid, wherein said sequences flank a variable nucleic acid sequence of said bioagent in said environmental sample; producing an amplification product of said variable nucleic acid sequence; determining a first molecular mass of said amplification product; and comparing said first molecular mass to the molecular masses of known bioagents, thereby identifying the unknown

2. The method of claim 1 wherein said sequences to which said at least one pair of **oligonucleotide primers** hybridize are highly conserved.
3. The method of claim 1 wherein said sequences to which said at least one pair of **oligonucleotide primers** hybridize are highly conserved across at least two species.
4. The method of claim 1 further comprising the step of isolating a nucleic acid from said environmental sample prior to contacting said nucleic acid with said at least one pair of **oligonucleotide primers**, wherein the comparing step further comprises comparing a base-pair count resulting from a translation of the corresponding molecular mass, and wherein a master database of molecular masses of known bioagents further includes a translation of said molecular masses of known bioagents to corresponding base-pair counts of each known bioagent resulting from a specific **primer** pair set and comparing the base-pair count of said unknown bioagent against the obtained base-pair count of known bioagents for the selected **primer** pair set for determining the identity of said unknown bioagent in said environmental sample.
5. The method of claim 4 further comprising the step of reconciling the database of molecular masses of known bioagents with the master database of molecular masses of known bioagents.
6. The method of claim 1 wherein said bioagent is a bacterium, parasite, fungi, virus, cell or spore.
7. The method of claim 1 wherein said environmental sample is a water sample, air sample, or land sample.
8. The method of claim 7 wherein said water sample is obtained from a lake, river, ocean, stream, water treatment system, rainwater, groundwater, water table, reservoir, well, or bottled water.
9. The method of claim 7 wherein said air sample is obtained from a ventilation system, airplane cabin, school, hospital, or mass transit location.
10. The method of claim 9 wherein said mass transit location is a subway, train station, or airport.
11. The method of claim 1 wherein said amplification product is ionized by electrospray ionization, matrix assisted laser desorption or fast atom bombardment prior to molecular mass determination.
12. The method of claim 1 wherein said molecular mass is determined by mass spectrometry.
13. The method of claim 5 wherein said master database of molecular masses of known bioagents and the database of molecular masses of known bioagents are reconciled over a network.
14. The method of claim 4 wherein the identity is determined by statistically correlating the molecular mass of the unknown bioagent with at least one molecular mass of said master database.
15. A database having cell-data positional significance comprising at least a first table of a plurality of data-containing cells, said first table organized into at least a first row and a second row, each row having columns and data-containing cells; and wherein said data-containing cells have an alignment with at least one other row for differentiating aligned from non-aligned data-containing cells, and wherein said differentiation in alignment of said data-containing cells designates a structural feature of a polymer present in an environmental sample.
16. The database according to claim 15 wherein said alignment is a vertical alignment according to base pair homology along a linear segment within each polymer.
17. The database according to claim 15 wherein said vertical alignment further aligns cell-data according to inter-species conserved regions.
18. The database according to claim 15 wherein the structural feature is a bulge or a loop.
19. The database according to claim 15 wherein the polymer is an RNA.
20. The method of claim 15 wherein said environmental sample is a water sample, air sample, or land sample.

lake, river, ocean, stream, water treatment system, rainwater, groundwater, water table, reservoir, well, or bottled water.

22. The method of claim 20 wherein said air sample is obtained from a ventilation system, airplane cabin, school, hospital, or mass transit location.

23. The method of claim 22 wherein said mass transit location is a subway, train station, or airport.

24. A service providing information related to a bioagent in an environmental sample comprising: providing a dimensional master database for storing a molecular mass, an identity and a detail corresponding to a plurality of known bioagents and, said master database storing the molecular mass, the identity and the detail for a plurality of known bioagents; interrogating the master database with an identification request of an unknown bioagent in said environmental sample to generate a response; and delivering said response from the master database to a requester.

25. The service according to claim 24 wherein the molecular mass is of a selected portion of the known bioagent, the identity comprises at least a geographic origin and a name for the known bioagent, and the detail comprises at least a treatment.

26. The service according to claim 24 wherein the request comprises a symptomatology and the identification comprises a recommended pair of **primers** for hybridizing to sequences of nucleic acid flanking a variable nucleic acid sequence of the unknown bioagent, and said pair of **primers** are hybridized to the sequences of nucleic acid flanking a variable nucleic acid sequence of the unknown bioagent.

27. The service according to claim 26 wherein the nucleic acid sequence of the unknown bioagent between said pair of **primers** defines the selected portion of both the known bioagents and the unknown bioagent.

28. The service according to claim 27 wherein the response is delivered through a network.

29. The service according to claim 27 wherein the request comprises a molecular mass of the unknown bioagent for the selected portion and where the response generated thereto comprises a set of molecular masses for analogous selected portions of known bioagents, and said set comprising at least one molecular mass from the master database.

30. The service according to claim 28 wherein the network is a local area network.

31. The service according to claim 28 wherein the network is a wide area network.

32. The service according to claim 29 wherein the network is the internet.

33. A method of determining a geographical origin of a selected bioagent in an environmental sample using a database of molecular masses of known bioagents comprising: contacting nucleic acid from said selected bioagent in said environmental sample with at least one pair of **oligonucleotide primers** which hybridize to sequences of said nucleic acid, wherein said sequences flank a variable nucleic acid sequence of the bioagent in said environmental sample; producing an amplification product of said variable nucleic acid sequence; determining a first molecular mass of said amplification product; and comparing said first molecular mass to the molecular masses of known bioagents for determining a geographic origin of said selected bioagent, said comparison determining an identity and a geographic origin of said selected bioagent in said environmental sample.

34. The method of claim 33 wherein said sequences to which said at least one pair of **oligonucleotide primers** hybridize are highly conserved.

35. The method of claim 33 wherein said sequences to which said at least one pair of **oligonucleotide primers** hybridize are highly conserved across species.

36. The method of claim 33 further comprising the step of isolating a nucleic acid from said selected bioagent prior to contacting said nucleic acid with said at least one pair of **oligonucleotide primers**, wherein the comparing step further comprises interrogating a master database of molecular masses of known bioagents for obtaining molecular masses of known bioagents and comparing the molecular mass of said selected bioagent against the obtained molecular masses of known

37. The method of claim 36 further comprising the step of reconciling the database of molecular masses of known bioagents with the master database of molecular masses of known bioagents.

38. The method of claim 33 wherein said bioagent is a bacterium, parasite, fungi, virus, ell or spore.

39. The method of claim 33 wherein said environmental sample is a water sample, air sample, or land sample.

40. The method of claim 39 wherein said water sample is obtained from a lake, river, ocean, stream, water treatment system, rainwater, groundwater, water table, reservoir, well, or bottled water.

41. The method of claim 39 wherein said air sample is obtained from a ventilation system, airplane cabin, school, hospital, or mass transit location.

42. The method of claim 41 wherein said mass transit location is a subway, train station, or airport.

43. The method of claim 33 wherein said amplification product is ionized by electrospray ionization, matrix assisted laser desorption or fast atom bombardment prior to molecular mass determination.

44. The method of claim 33 wherein said molecular mass is determined by mass spectrometry.

45. The method of claim 36 wherein said master database of molecular masses of known bioagents and the database of molecular masses of known bioagents are reconciled over a network.

46. The method of claim 36 wherein the origin comprises a statistical group of matching molecular masses and the geographic origin corresponding thereto.

L5 ANSWER 30 OF 112 USPTAFULL on STN

2003:312155 Novel antigen binding molecules for therapeutic, diagnostic, prophylactic, enzymatic, industrial, and agricultural applications, and methods for generating and screening thereof.
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Diversa Corporation, San Diego, CA, UNITED STATES, 92121 (U.S. corporation)
US 2003219752 A1 20031127

APPLICATION: US 2002-151469 A1 20020517 (10)

PRIORITY: US 2001-300381P 20010517 (60)

US 2001-300907P 20010625 (60)

US 1995-8311P 19951207 (60)

US 1995-8316P 19951207 (60)

US 1995-8311P 19951207 (60)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention is directed to methods for generating sets, or libraries, of nucleic acids encoding antigen-binding sites, such as antibodies, antibody domains or other fragments, including single and double stranded antibodies, major histocompatibility complex (MHC) molecules, T cell receptors (TCRs), and the like. This invention provides methods for generating variant antigen binding sites, e.g., antibodies and specific domains or fragments of antibodies (e.g., Fab or Fc domains), by altering template nucleic acids including by saturation mutagenesis, synthetic ligation reassembly, or a combination thereof. In one aspect, invention provides methods for generating all human or humanized antibodies and evolving them to achieve optimized properties related to stability, duration, expression, production, enzymatic activity, affinity, avidity, localization, and other immunological properties. Polypeptides generated by these methods can be analyzed using a novel capillary array platform, which provides unprecedented ultra-high throughput screening.

CLM What is claimed is:

1. A method for producing a library of nucleic acids encoding a plurality of modified antigen binding sites, wherein the modified antigen binding sites are derived from a first nucleic acid comprising a sequence encoding a first antigen binding site, the method comprising:
(a) providing a first nucleic acid encoding a first antigen binding site; (b) providing a set of mutagenic **oligonucleotides** that encode naturally-occurring amino acid variants at a plurality of targeted codons in the first nucleic acid; and, (c) using the set of mutagenic **oligonucleotides** to generate a set of antigen binding site-encoding variant nucleic acids encoding a range of amino acid variations at each amino acid codon that was mutagenized, thereby producing a library of nucleic acids encoding a plurality of modified antigen binding sites.

2. The method of claim 1, wherein step (b) provides a set of mutagenic **oligonucleotides** that encode all nineteen naturally-occurring amino acid variants for each targeted codon, thereby generating all 19 possible natural amino acid changes at each amino acid codon mutagenized.
3. The method of claim 1, further comprising expressing the set of variant antigen binding site-encoding nucleic acids such that antigen binding site-encoding polypeptides encoded by the variant nucleic acids are expressed.
4. The method of claim 1, wherein the set of mutagenic **oligonucleotides** comprises a 19-fold degenerate mutagenic **oligonucleotide** for each codon to be mutagenized, wherein each of the 19-fold degenerate mutagenic **oligonucleotides** comprises a homologous first sequence and a degenerate triplet second sequence.
5. The method of claim 1, wherein the antigen binding site comprises a single stranded antigen binding polypeptide, a Fab fragment, an Fc fragment, a Fd fragment, a F(ab')₂ fragment, a Fv fragment or a complementarity determining region (CDR).
6. The method of claim 5, wherein the antigen binding site polypeptide further comprises an antibody polypeptide.
7. The method of claim 1, wherein the antigen binding site polypeptide further comprises an antigen binding site of a T cell receptor (TCR).
8. The method of claim 7, wherein the antigen binding site polypeptide further comprises a T cell receptor (TCR).
9. The method of claim 1, wherein the antigen binding site polypeptide further comprises an antigen binding site of a major histocompatibility complex (MHC) molecule.
10. The method of claim 9, wherein the antigen binding site polypeptide further comprises a major histocompatibility complex (MIC) molecule.
11. The method of claim 10, wherein the major histocompatibility complex (MRC) molecule comprises a Class I molecule.
12. The method of claim 10, wherein the major histocompatibility complex (MHC) molecule comprises a Class II molecule.
13. The method of claim 1, wherein the nucleic acid of step (a) is derived from a nucleic acid encoding a mammalian polypeptide.
14. The method of claim 13, wherein the mammalian polypeptide comprises a human polypeptide.
15. The method of claim 13, wherein the mammalian polypeptide is selected from the group consisting of an antibody, a T cell receptor, a Class I MHC molecule and a Class II MHC molecule.
16. The method of claim 1, wherein the nucleic acid of step (a) is derived from a human nucleic acid encoding an antigen binding site.
17. The method of claim 16, wherein the nucleic acid of step (a) is derived from a phage comprising a human nucleic acid sequence encoding an antigen binding site, wherein the phage expresses the antigen binding site.
18. The method of claim 16, wherein the nucleic acid of step (a) is derived from a non-human mammal comprising a human nucleic acid sequence encoding an antigen binding site, wherein the non-human mammal expresses the antigen binding site.
19. The method of claim 18, wherein the non-human mammal is a transgenic non-human mammal.
20. The method of claim 19, wherein the transgenic non-human mammal is a mouse.
21. The method of claim 1, wherein at least two amino acid codons in the antigen binding site are mutagenized.
22. The method of claim 21, wherein all the amino acid codons in the antigen binding site are mutagenized.
23. The method of claim 6, wherein all the amino acid codons in the antibody polypeptide are mutagenized.

cell receptor (TCR) are mutagenized.

25. The method of claim 10, wherein all the amino acid codons in the MHC molecule are mutagenized.

26. The method of claim 1, wherein a degenerate mutagenic **oligonucleotide** comprises a first homologous sequence, a degenerate triplet second sequence, and a third homologous sequence.

27. The method of claim 1, wherein each degenerate **oligonucleotide** comprises a first homologous sequence, a plurality of degenerate triplets second sequences, and a third homologous sequence.

28. The method of claim 3, further comprising screening the expressed antigen binding site polypeptide for its ability to specifically bind an antigen.

29. The method of claim 28, comprising screening the expressed antigen binding site polypeptide for its ability to specifically bind an antigen capable of being specifically bound by the first antigen binding site polypeptide.

30. The method of claim 29, comprising identifying an antigen binding site variant by its increased antigen binding affinity or antigen binding specificity as compared to the affinity or specificity of the first antigen binding site to the antigen.

31. The method of claim 29, comprising identifying an antigen binding site variant by its decreased antigen binding affinity or antigen binding specificity as compared to the affinity or specificity of the first antigen binding site to the antigen.

32. The method of claim 1, further comprising mutagenizing the first nucleic acid of step (a) by a method comprising an optimized directed evolution system.

33. The method of claim 1, further comprising mutagenizing the first nucleic acid of step (a) by a method comprising a synthetic ligation reassembly.

34. The method of claim 3, comprising screening the expressed antigen binding site polypeptide for its ability to specifically bind an antigen by a method comprising expression of the expressed antigen binding site polypeptide in a solid phase.

35. The method of claim 34, comprising screening the expressed antigen binding site polypeptide for its ability to specifically bind an antigen by a method comprising a capillary array.

36. The method of claim 34, comprising screening the expressed antigen binding site polypeptide for its ability to specifically bind an antigen by a method comprising a double-orificed container.

37. The method of claim 36, wherein the double-orificed container comprises a double-orificed capillary array.

38. The method of claim 37, wherein the double-orificed capillary array is a GIGAMATRIX.TM. capillary array.

39. The method of claim 34, comprising screening the expressed antigen binding site polypeptide for its ability to specifically bind an antigen by a method comprising use of an ELISA.

40. The method of claim 3, comprising screening the expressed antigen binding site polypeptide for its ability to specifically bind an antigen by a method comprising phage display of the antigen binding site polypeptide.

41. The method of claim 3, comprising screening the expressed antigen binding site polypeptide for its ability to specifically bind an antigen by a method comprising expression of the expressed antigen binding site polypeptide in a liquid phase.

42. The method of claim 3, comprising screening the expressed antigen binding site polypeptide for its ability to specifically bind an antigen by a method comprising ribosome display of the antigen binding site polypeptide.

43. The method of claim 1, wherein the set of progeny antigen binding site-encoding variant nucleic acids is generated by amplifying the nucleic acid of step (a) by a polymerase-based amplification using a plurality of **oligonucleotides**.

44. The method of claim 43, wherein the amplification comprises a **polymerase chain reaction (PCR)**.

45. A library of nucleic acids encoding a plurality of modified antigen binding sites, wherein the modified antigen binding sites are derived from a first nucleic acid comprising a sequence encoding a first antigen binding site, made by a method comprising the following steps: (a) providing a first nucleic acid encoding a first antigen binding site; (b) providing a set of mutagenic **oligonucleotides** that encode naturally-occurring amino acid variants at a plurality of targeted codons in the first nucleic acid; and, (c) using the set of mutagenic **oligonucleotides** to generate a set of antigen binding site-encoding variant nucleic acids encoding a range of amino acid variations at each amino acid codon that was mutagenized, thereby producing a library of nucleic acids encoding a plurality of modified antigen binding sites.

46. A method for producing from a library of variant antibodies from a template antibody, the method comprising: (a) providing a first nucleic acid encoding the template antibody; (b) providing a set of mutagenic **oligonucleotides** that encode naturally-occurring amino acid variants at a plurality of targeted codons in the first nucleic acid; and, c) using the set of mutagenic **oligonucleotides** to generate a set of antibody-encoding variant nucleic acids encoding a range of amino acid variations at each amino acid codon that was mutagenized, thereby producing a library of nucleic acids encoding a plurality of variant antibodies.

47. The method of claim 46, wherein step (b) provides a set of mutagenic **oligonucleotides** that encode all nineteen naturally-occurring amino acid variants for each targeted codon, thereby generating all 19 possible natural amino acid changes at each amino acid codon mutagenized.

48. The method of claim 46, wherein the antibody is selected from the group consisting of polypeptides comprising a Fab fragment, an Fd fragment, an Fc fragment, a F(ab')₂ fragment, a Fv fragment and a complementarity determining region (CDR).

49. The method of claim 46, wherein the plurality of **oligonucleotides** comprises a degenerate **oligonucleotide** for each codon to be mutagenized, wherein each of the degenerate **oligonucleotides** comprises a homologous first sequence and a degenerate triplet second sequence.

50. The method of claim 46, wherein the set of progeny polynucleotides encoding antibodies is generated by amplifying the nucleic acid of step (a) using a plurality of **oligonucleotides**.

51. A library of variant antibodies derived from a template antibody made by a method comprising the following steps: (a) providing a first nucleic acid encoding the template antibody; (b) providing a set of mutagenic **oligonucleotides** that encode naturally-occurring amino acid variants at a plurality of targeted codons in the first nucleic acid; and, c) using the set of mutagenic **oligonucleotides** to generate a set of antibody-encoding variant nucleic acids encoding a range of amino acid variations at each amino acid codon that was mutagenized, thereby producing a library of nucleic acids encoding a plurality of variant antibodies.

52. A method for producing from a library of variant T cell receptors (TCRs) from a template T cell receptor (TCR), the method comprising: (a) providing a first nucleic acid encoding the template T cell receptor; (b) providing a set of mutagenic **oligonucleotides** that encode naturally-occurring amino acid variants at a plurality of targeted codons in the first nucleic acid; and, c) using the set of mutagenic **oligonucleotides** to generate a set of T cell receptor (TCR)-encoding variant nucleic acids encoding a range of amino acid variations at each amino acid codon that was mutagenized, thereby producing a library of nucleic acids encoding a plurality of variant T cell receptors (TCRs).

53. A library of variant T cell receptors (TCRs) derived from a template T cell receptor (TCR) made by a method comprising the following steps: (a) providing a first nucleic acid encoding the template T cell receptor; (b) providing a set of mutagenic **oligonucleotides** that encode naturally-occurring amino acid variants at a plurality of targeted codons in the first nucleic acid; and, c) using the set of mutagenic **oligonucleotides** to generate a set of T cell receptor (TCR)-encoding variant nucleic acids encoding a range of amino acid variations at each amino acid codon that was mutagenized, thereby producing a library of nucleic acids encoding a plurality of variant T cell receptors (TCRs).

histocompatibility complex (MHC) molecules from a template major histocompatibility complex (MHC) molecule, the method comprising: (a) providing a first nucleic acid encoding the template major histocompatibility complex (MHC) molecule; (b) providing a set of mutagenic **oligonucleotides** that encode naturally-occurring amino acid variants at a plurality of targeted codons in the first nucleic acid; and, c) using the set of mutagenic **oligonucleotides** to generate a set of major histocompatibility complex (MHC) molecule-encoding variant nucleic acids encoding a range of amino acid variations at each amino acid codon that was mutagenized, thereby producing a library of nucleic acids encoding a plurality of variant major histocompatibility complex (MHC) molecules.

55. A library of variant major histocompatibility complex (MHC) molecules derived from a template major histocompatibility complex (MHC) molecule made by a method comprising the following steps: (a) providing a first nucleic acid encoding the template major histocompatibility complex (MHC) molecule; (b) providing a set of mutagenic **oligonucleotides** that encode naturally-occurring amino acid variants at a plurality of targeted codons in the first nucleic acid; and, c) using the set of mutagenic **oligonucleotides** to generate a set of major histocompatibility complex (MHC) molecule-encoding variant nucleic acids encoding a range of amino acid variations at each amino acid codon that was mutagenized, thereby producing a library of nucleic acids encoding a plurality of variant major histocompatibility complex (MHC) molecules.

56. A method of making a set of nucleic acids encoding a set of antigen binding site variants comprising the steps of: (a) providing a template nucleic acid encoding an antigen-binding polypeptide; (b) providing a plurality of **oligonucleotides** that encode all nineteen naturally-occurring amino acid variants at a single amino acid residue of the antigen-binding polypeptide; and, (c) generating a set of progeny antigen binding site-encoding variant nucleic acids encoding a non-stochastic range of single amino acid substitutions at each amino acid codon that was mutagenized, whereby all 19 possible natural amino acid changes are generated at each amino acid codon mutagenized, thereby making a set of nucleic acids encoding a set of antigen binding site variants.

57. The method of claim 56, further comprising expressing the set of progeny antigen binding site-encoding polynucleotides such that antigen binding site-encoding polypeptides encoded by the progeny polynucleotides are expressed.

58. The method of claim 56, wherein the plurality of **oligonucleotides** comprises a set of degenerate **oligonucleotides** and each of the degenerate **oligonucleotides** comprises a homologous first sequence and a degenerate triplet second sequence.

59. The method of claim 56, wherein the antigen binding site-encoding polypeptide comprises a single stranded antigen binding polypeptide.

60. The method of claim 56, wherein the antigen binding site-encoding polypeptide comprises an antibody polypeptide.

61. The method of claim 56, wherein the antigen binding site-encoding polypeptide comprises an antigen binding site of a T cell receptor (TCR).

62. The method of claim 61, wherein the antigen binding site-encoding polypeptide further comprises a T cell receptor (TCR).

63. The method of claim 56, wherein the antigen binding site-encoding polypeptide comprises an antigen binding site of a major histocompatibility complex (MHC) molecule.

64. The method of claim 63, wherein the antigen binding site-encoding polypeptide further comprises a major histocompatibility complex (MHC) molecule.

65. The method of claim 56, wherein the nucleic acid of step (a) is derived from a nucleic acid encoding a mammalian antibody polypeptide.

66. The method of claim 65, wherein the nucleic acid of step (a) is derived from a human nucleic acid.

67. The method of claim 56, wherein at least two amino acid codons in the antigen binding site are mutagenized and a set of degenerate **oligonucleotides** that encode all nineteen naturally-occurring amino acid variants are provided for each amino acid codon mutagenized.

68. The method of claim 56, wherein all the amino acid codons in the

oligonucleotides that encode all nineteen naturally-occurring amino acid variants are provided for each amino acid codon mutagenized.

69. The method of claim 60, wherein all the amino acid codons in the antibody polypeptide are mutagenized.

70. The method of claim 61, wherein all the amino acid codons in the antigen binding site of the T cell receptor (TCR) are mutagenized.

71. The method of claim 63, wherein all the amino acid codons in the antigen binding site of the major histocompatibility complex (MHC) molecule are mutagenized.

72. The method of claim 56, wherein a degenerate **oligonucleotide** comprises a first homologous sequence, a degenerate triplet second sequence, and a homologous third sequence.

73. The method of claim 56, wherein each degenerate **oligonucleotide** comprises a first homologous sequence, a degenerate triplet second sequence, and a homologous third sequence.

74. The method of claim 57, further comprising screening an expressed antigen binding site-encoding polypeptide for its ability to specifically bind an antigen.

75. The method of claim 57, comprising screening the expressed antigen binding site-encoding polypeptide for its ability to specifically bind an antigen capable of being specifically bound by the first antigen binding site.

76. The method of claim 75, comprising identifying an antigen binding site variant by its increased antigen binding affinity or antigen binding specificity to the antigen as compared to the affinity or specificity of the antigen binding site encoded by the nucleic acid of step (a).

77. The method of claim 56, further comprising mutagenizing the template nucleic acid by a method comprising an optimized directed evolution system.

78. The method of claim 56, further comprising mutagenizing the template nucleic acid by a method comprising a synthetic ligation reassembly.

79. The method of claim 56, comprising screening the expressed antigen binding site-encoding polypeptide for its ability to specifically bind an antigen by a method comprising a capillary array.

80. The method of claim 56, comprising screening the expressed antigen binding site-encoding polypeptide for its ability to specifically bind an antigen by an ELISA.

81. The method of claim 56, wherein the set of variant nucleic acids is generated by performing amplification reactions on the nucleic acid of step (a) using the set of **oligonucleotides** to generate a set of variant nucleic acids encoding nineteen amino acid substitution variants at a single amino acid residue of the antigen-binding polypeptide.

82. The method of claim 81, wherein the amplification comprises a polymerase-based amplification.

83. The method of claim 82, wherein polymerase-based amplification comprises a **polymerase chain reaction (PCR)**.

84. The method of claim 56, wherein the set of variant nucleic acids comprises 10^{10} members.

85. The method of claim 56, wherein the set of variant nucleic acids comprises 10^5 members.

86. The method of claim 56, wherein the set of variant nucleic acids comprises 10^3 members.

87. A method of making a set of antibody variants comprising the steps of: (a) providing a nucleic acid encoding an antibody; (b) providing a plurality of **oligonucleotides**; (c) generating a non-stochastic range of single amino acid substitutions at each amino acid codon, whereby all 19 possible natural amino acid changes are generated at each amino acid codon mutagenized, thereby generating a set of variant nucleic acids; and, (d) expressing the set of variant nucleic acids such that the antibody variants encoded by the variant nucleic acids are expressed.

88. The method of claim 87, wherein the antibody is selected from the

fragment, an Fc fragment, a F(ab')₂ fragment, a Fv fragment and a complementarity determining region (CDR).

89. The method of claim 87, wherein the plurality of **oligonucleotides** comprises a set of degenerate **oligonucleotides** that encode all nineteen naturally-occurring amino acid variants at a single amino acid residue of the antibody, wherein each of the degenerate **oligonucleotides** comprises a homologous first sequence and a degenerate triplet second sequence.

90. The method of claim 87, wherein generating a non-stochastic range of single amino acid substitutions comprises performing amplification reactions on the nucleic acid of step (a) using the set of **oligonucleotides** to generate a set of variant nucleic acids encoding nineteen amino acid substitution variants at a single amino acid residue of the antibody.

91. A method of identifying a variant of an antigen binding site comprising the steps of: (a) providing a nucleic acid encoding an antigen binding site; (b) providing a set of **oligonucleotides** that encode all nineteen naturally-occurring amino acid variants at all residues of the antigen-binding site; (c) incorporating the sequence of the **oligonucleotides** of step (b) into the nucleic acid of step (a) to generate a set of variant nucleic acids encoding nineteen amino acid substitution variants at each residue of the antigen binding site; (d) expressing each of the variant nucleic acids as polypeptides and measuring the variant's affinity to the antigen; and, (e) identifying a variant of the antigen binding site by its increased or decreased antigen binding specificity as compared to the antigen binding affinity of the antigen binding site encoded by the nucleic acid of step (a).

92. The method of claim 91, wherein the variant nucleic acids are expressed using in vitro transcription/translation.

93. The method of claim 91, wherein the variant nucleic acids are expressed using phage display.

94. The method of claim 91, wherein the variant nucleic acids are expressed using f2o ribosome display.

95. The method of claim 91, wherein the variant nucleic acids are expressed using a double orificed container.

96. The method of claim 95, wherein the variant nucleic acids are expressed using a double orificed capillary array.

97. The method of claim 91, wherein the set of **oligonucleotides** comprises a set of degenerate **oligonucleotides** that encode all nineteen naturally-occurring amino acid variants at a single amino acid residue of the antibody, wherein each of the degenerate **oligonucleotides** comprises a homologous first sequence and a degenerate triplet second sequence.

98. The method of claim 91, wherein the antigen binding site comprises an antibody.

99. The method of claim 98, wherein the antibody is selected from the group consisting of polypeptides comprising a Fab fragment, an Fd fragment, an Fc fragment, a F(ab')₂ fragment, a Fv fragment and a complementarity determining region (CDR).

100. The method of claim 91, wherein the antigen binding site comprises an antigen binding site of a T cell receptor.

101. The method of claim 91, wherein the antigen binding site comprises an antigen binding site of a major histocompatibility complex molecule.

102. The method of claim 91, wherein incorporating the sequence of the **oligonucleotides** of step (b) into the nucleic acid of step (a) is accomplished by an amplification reaction using the **oligonucleotides** as **primers**.

L5 ANSWER 31 OF 112 USPTAFULL on STN

2003:294272 Non-stochastic generation of genetic vaccines.

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US 2003207287 A1 20031106

APPLICATION: US 2002-223507 A1 20020819 (10)

PRIORITY: US 1995-8311P 19951207 (60)

US 1995-8316P 19951207 (60)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

non-stochastic methods of directed evolution (DirectEvolution.TM.). These methods include non-stochastic polynucleotide site-saturation mutagenesis (Gene Site Saturation Mutagenesis.TM.) and non-stochastic polynucleotide reassembly (GeneReassembly.TM.). Through use of the claimed methods, vectors can be obtained which exhibit increased efficacy for use as genetic vaccines. Vectors obtained by using the methods can have, for example, enhanced antigen expression, increased uptake into a cell, increased stability in a cell, ability to tailor an immune response, and the like.

CLM What is claimed is:

1. A method for obtaining an immunomodulatory polynucleotide that has an optimized modulatory effect on an immune response, or encodes a polypeptide that has an optimized modulatory effect on an immune response, the method comprising: creating a library of non-stochastically generated progeny polynucleotides from a parental polynucleotide set; wherein optimization can thus be achieved using one or more of the directed evolution methods as described herein in any combination, permutation and iterative manner; whereby these directed evolution methods include the introduction of mutations by non-stochastic methods, including by "gene site saturation mutagenesis" as described herein; and whereby these directed evolution methods also include the introduction mutations by non-stochastic polynucleotide reassembly methods as described herein; including by synthetic ligation polynucleotide reassembly as described herein.

2. The method of claim 1, wherein said optimized modulatory effect on an immune response is induced by a genetic vaccine vector. Screening

3. A method for obtaining an immunomodulatory polynucleotide that has an optimized modulatory effect on an immune response, or encodes a polypeptide that has an optimized modulatory effect on an immune response, the method comprising: screening a library of non-stochastically generated progeny polynucleotides to identify an optimized non-stochastically generated progeny polynucleotide that has, or encodes a polypeptide that has, a modulatory effect on an immune response; wherein the optimized non-stochastically generated polynucleotide or the polypeptide encoded by the non-stochastically generated polynucleotide exhibits an enhanced ability to modulate an immune response compared to a parental polynucleotide from which the library was created.

4. The method of claim 3, wherein said optimized modulatory effect on an immune response is induced by a genetic vaccine vector. Evolution & Screening

5. A method for obtaining an immunomodulatory polynucleotide that has an optimized modulatory effect on an immune response, or encodes a polypeptide that has an optimized modulatory effect on an immune response, the method comprising: a) creating a library of non-stochastically generated progeny polynucleotides from a parental polynucleotide set; and b) screening the library to identify an optimized non-stochastically generated progeny polynucleotide that has, or encodes a polypeptide that has, a modulatory effect on an immune response induced by a genetic vaccine vector; wherein the optimized non-stochastically generated polynucleotide or the polypeptide encoded by the non-stochastically generated polynucleotide exhibits an enhanced ability to modulate an immune response compared to a parental polynucleotide from which the library was created; whereby optimization can thus be achieved using one or more of the directed evolution methods as described herein in any combination, permutation, and iterative manner; whereby these directed evolution methods include the introduction of point mutations by non-stochastic methods, including by "gene site saturation mutagenesis" as described herein; and whereby these directed evolution methods also include the introduction mutations by non-stochastic polynucleotide reassembly methods as described herein; including by synthetic ligation polynucleotide reassembly as described herein.

6. The method of claim 5, wherein said optimized modulatory effect on an immune response is induced by a genetic vaccine vector.

7. The method of any of claims 1-6, wherein the optimized non-stochastically generated polynucleotide is incorporated into a genetic vaccine vector.

8. The method of any of claims 1-6, wherein the optimized non-stochastically generated polynucleotide, or a polypeptide encoded by the optimized non-stochastically generated polynucleotide, is administered in conjunction with a genetic vaccine vector.

9. The method of any of claims 1-6, wherein the library of non-stochastically generated progeny polynucleotides is created by a

oligonucleotide-directed saturation mutagenesis, and any combination, permutation and iterative manner.

10. The method of any of claims 1-6, wherein the optimized non-stochastically generated polynucleotide that has a modulatory effect on an immune response is obtained by: a) non-stochastically reassembling at least two parental template polynucleotide, each of which is, or encodes a molecule that is, involved in modulating an immune response; wherein the first and second parental templates differ from each other in two or more nucleotides, to produce a library of non-stochastically generated polynucleotides; and b) screening the library to identify at least one optimized non-stochastically generated polynucleotide that exhibits, either by itself or through the encoded molecule, an enhanced ability to modulate an immune response in comparison to a parental polynucleotide from which the library was created.

11. The method of claim 10, wherein the method further comprises the steps of: c) subjecting a working optimized non-stochastically generated polynucleotide to a further round of non-stochastic reassembly with at least one additional polynucleotide, which is the same or different from the first and second polynucleotides, to produce a further working library of recombinant polynucleotides; d) screening the further working library to identify at least one further optimized non-stochastically generated polynucleotide that exhibits an enhanced ability to modulate an immune response in comparison to a parental polynucleotide from which the library was created; and e) optionally repeating c) and d) as necessary, until a desirable further optimized non-stochastically generated polynucleotide that exhibits an enhanced ability to modulate an immune response than a form of the nucleic acid from which the library was created.

12. The method of any of claims 1-6, wherein the optimized non-stochastically generated polynucleotide encodes a polypeptide that can interact with a cellular receptor involved in mediating an immune response; wherein the polypeptide acts as an agonist or antagonist of the receptor.

13. The method of claim 12, wherein the cellular receptor is a macrophage scavenger receptor.

14. The method of claim 12, wherein the cellular receptor is selected from the group consisting of a cytokine receptor and a chemokine receptor.

15. The method of claim 14, wherein the chemokine receptor is CCR6.

16. The method of claim 12, wherein the polypeptide mimics the activity of a natural ligand for the receptor but does not induce immune reactivity to said natural ligand.

17. The method of claim 12, wherein the library is screened by: i) expressing the non-stochastically generated progeny polynucleotides so that the encoded polypeptides are produced as fusions with a protein displayed on the surface of a replicable genetic package; ii) contacting the replicable genetic packages with a plurality of cells that display the receptor; and iii) identifying cells that exhibit a modulation of an immune response mediated by the receptor.

18. The method of claim 17, wherein the replicable genetic package is selected from the group consisting of a bacteriophage, a cell, a spore, and a virus.

19. The method of claim 18, wherein the replicable genetic package is an M13 bacteriophage and the protein is encoded by geneIII or geneVIII.

20. The method of claim 12, which method further comprises introducing the optimized non-stochastically generated polynucleotide into a genetic vaccine vector and administering the vector to a mammal, wherein the peptide or polypeptide is expressed and acts as an agonist or antagonist of the receptor.

21. The method of claim 12, which method further comprises producing the polypeptide encoded by the optimized non-stochastically generated polynucleotide and introducing the polypeptide into a mammal in conjunction with a genetic vaccine vector.

22. The method of claim 12, wherein the optimized non-stochastically generated polynucleotide is inserted into an antigen-encoding nucleotide sequence of a genetic vaccine vector.

23. The method of claim 22, wherein the optimized non-stochastically

encodes an M-loop of an HBsAg polypeptide.

24. The method of any of claims 1-6, wherein the optimized non-stochastically generated polynucleotide comprises a nucleotide sequence rich in unmethylated CpG.

25. The method of any of claims 1-6, wherein the optimized non-stochastically generated polynucleotide encodes a polypeptide that inhibits an allergic reaction.

26. The method of claim 25, wherein the polypeptide is selected from the group consisting of interferon- α , interferon- γ , IL-10, IL-12, an antagonist of IL-4, an antagonist of IL-5, and an antagonist of IL-13.

27. The method of 1, wherein the optimized recombinant polynucleotide encodes an antagonist of IL-10.

28. The method of claim 27, wherein the antagonist of IL-10 is soluble or defective IL-10 receptor or IL-20/MDA-7.

29. The method of any of claims 1-6, wherein the optimized non-stochastically generated polynucleotide encodes a co-stimulator.

30. The method of claim 29, wherein the co-stimulator is B7-1 (CD80) or B7-2 (CD86) and the screening step involves selecting variants with altered activity through CD28 or CTLA-4.

31. The method of claim 29, wherein the co-stimulator is CD1, CD40, CD154 (ligand for CD40) or CD150 (SLAM).

32. The method of claim 29, wherein the co-stimulator is a cytokine.

33. The method of claim 32, wherein the cytokine is selected from the group consisting of IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, GM-CSF, G-CSF, TNF- α , IFN- α , IFN- γ , and IL-20 (MDA-7).

34. The method of 33, wherein the library of non-stochastically generated polynucleotides is screened by testing the ability of cytokines encoded by the non-stochastically generated polynucleotides to activate cells which contain a receptor for the cytokine.

35. The method of claim 34, wherein the cells contain a heterologous nucleic acid that encodes the receptor for the cytokine.

36. The method of 33, wherein the cytokine is interleukin-12 and the screening is performed by: growing mammalian cells which contain the genetic vaccine vector in a culture medium; and detecting whether T cell proliferation or T cell differentiation is induced by contact with the culture medium.

37. The method of 33, wherein the cytokine is interferon- α and the screening is performed by: i) expressing the non-stochastically generated polynucleotides so that the encoded polypeptides are produced as fusions with a protein displayed on the surface of a replicable genetic package; ii) contacting the replicable genetic packages with a plurality of B cells; and iii) identifying phage library members that are capable of inhibiting proliferation of the B cells.

38. The method of claim 33, wherein the immune response of interest is differentiation of T cells to T_{H1} cells and the screening is performed by contacting a population of T cells with the cytokines encoded by the members of the library of recombinant polynucleotides and identifying library members that encode a cytokine that induces the T cells to produce IL-2 and interferon- γ .

39. The method of claim 32, wherein the cytokine encoded by the optimized non-stochastically generated polynucleotide exhibits reduced immunogenicity compared to a cytokine encoded by a non-optimized polynucleotide, and the reduced immunogenicity is detected by introducing a cytokine encoded by the non-stochastically generated polynucleotide into a mammal and determining whether an immune response is induced against the cytokine.

40. The method of claim 29, wherein the co-stimulator is B7-1 (CD80) or B7-2 (CD86) and the cell is tested for ability to costimulate an immune response.

41. The method of any of claims 1-6, wherein the optimized recombinant

42. The method of claim 41, wherein the cytokine antagonist is selected from the group consisting of a soluble cytokine receptor and a transmembrane cytokine receptor having a defective signal sequence.

43. The method of claim 41, wherein the cytokine antagonist is selected from the group consisting of AIL-1 OR and AIL-4R.

44. The method of any of claims 1-6, wherein the optimized non-stochastically generated polynucleotide encodes a polypeptide capable of inducing a predominantly T_{H1} immune response.

45. The method of any of claims 1-6, wherein the optimized non-stochastically generated polynucleotide encodes a polypeptide capable of inducing a predominantly T_{H2} immune response. Decreased Immune Response

46. The method of any of claims 1-6, wherein said optimized modulatory effect on an immune response is a decrease in an unwanted modulatory effect on an immune response; whereby application of the method can be used to generate a molecule having a decreased ability to elicit an immune response from a host recipient of said molecule, where said recipient can be a human or an animal host; and whereby application of the method can thus be used to generate a molecule having decreased antigenicity with respect to at least one host recipient of said molecule. Increased Immune Response

47. The method of any of claims 1-6, wherein said optimized modulatory effect on an immune response is an increase in a desirable modulatory effect on an immune response; whereby application of the method can be used to generate a molecule having an increased ability to elicit an immune response from a host recipient of said molecule, where said recipient can be a human or an animal host; and whereby application of the method can thus be used to generate a molecule having increased antigenicity with respect to at least one host recipient of said molecule. Decreased and Increased Immune Response

48. The method of any of claims 1-6, wherein said optimized modulatory effect on an immune response is both a decrease in a first unwanted modulatory effect on an immune response as well as an increase in a second desirable modulatory effect on an immune response; whereby application of the method can be used to generate a molecule having both a decreased ability to elicit a first immune response from a first host recipient of said molecule as well as an increased ability to elicit a second immune response from a second host recipient of said molecule; whereby the first and the second recipient hosts can be the same or different; whereby each of the first and the second recipient hosts can be a human or an animal host; and whereby application of the method can thus be used to generate a molecule having both a first decreased antigenicity with respect to at least one host recipient of said molecule and a second decreased antigenicity with respect to at least one host recipient of said molecule.

49. The method of claim 48, wherein said first and said second modulatory effect on an immune response are evolved for respectively a first and a second module on the same multimodule vaccine vector; whereby a module is exemplified by the following modules, as well as by a fragment derivative or analog thereof: an antigen coding sequence, a polyadenylation sequence, a sequence coding for a co-stimulatory molecule, a sequence coding for an inducible repressor or transactivator, a eukaryotic origin or replication, a prokaryotic origin of replication, a sequence coding for a prokaryotic marker,, and enhancer, a promoter, and operator, and an intron. Stability

50. The method of any of claims 1-6, wherein the optimized modulatory effect on an immune response is comprised of an increase in the stability of the immunomodulatory (IM) polynucleotide or polypeptide encoded thereby; whereby application of the method can be used to generate a molecule having an increased stability ex vivo, thus, for example, increasing shelf-life and/or ease of storage and/or length of time before expiration of activity upon storage; and whereby application of the method can also be used to generate a molecule having an increased stability in vivo upon administration to a host recipient, thus, for example, increasing resistance to digestive acids and/or increasing stability in the circulation and/or any other method of elimination or destruction by the host recipient. Human Vaccines

50. The method of any of claims 1-6, wherein the immunomodulatory (IM) polynucleotide or polypeptide encoded thereby; has an optimized modulatory effect on an immune response in a human host recipient; whereby application of the method can thus be used to generate an optimized genetic vaccine for human recipients. Animal Vaccines

51. The method of any of claims 1-6, wherein the immunomodulatory (IM) polynucleotide or polypeptide encoded thereby; has an optimized modulatory effect on an immune response in an animal host recipient; whereby application of the method can thus be used to generate an optimized genetic vaccine for animal recipients, including animals that are farmed or raised by man, animals that are not farmed or raised by man, domesticated animals, and non-domesticated animals. Accessory Molecules

52. A method for obtaining an optimized polynucleotide that encodes an accessory molecule that improves the transport or presentation of antigens by a cell, the method comprising: a) creating a library of non-stochastically generated polynucleotides by subjecting to optimization by non-stochastic directed evolution a parental polynucleotide set in which is encoded all or part of the accessory molecule; and b) screening the library to identify an optimized non-stochastically generated progeny polynucleotide that encodes a recombinant molecule that confers upon a cell an increased or decreased ability to transport or present an antigen on a surface of the cell compared to an accessory molecule encoded by template polynucleotides not subjected to the non-stochastic reassembly; whereby application of the method can thus be used to generate an optimized molecule for human recipients &/or animal recipients, including animals that are farmed or raised by man, animals that are not farmed or raised by man, domesticated animals, and non-domesticated animals; whereby optimization can thus be achieved using one or more of the directed evolution methods as described herein in any combination, permutation, and iterative manner; whereby these directed evolution methods include the introduction of point mutations by non-stochastic methods, including by "gene site saturation mutagenesis" as described herein; and whereby these directed evolution methods also include the introduction mutations by non-stochastic polynucleotide reassembly methods as described herein; including by synthetic ligation polynucleotide reassembly as described herein.

53. The method of claim 52, wherein the screening involves: i) introducing the library of non-stochastically generated polynucleotides into a genetic vaccine vector that encodes an antigen to form a library of vectors; introducing the library of vectors into mammalian cells; and ii) identifying mammalian cells that exhibit increased or decreased immunogenicity to the antigen.

54. The method of claim 52, wherein the accessory molecule comprises a proteasome or a TAP polypeptide.

55. The method of claim 52, wherein the accessory molecule comprises a cytotoxic T-cell inducing sequence.

56. The method of claim 55, wherein the cytotoxic T-cell inducing sequence is obtained from a hepatitis B surface antigen.

57. The method of claim 52, wherein the accessory molecule comprises an immunogenic agonist sequence. Plant Expression

58. A method for obtaining an immunomodulatory polynucleotide that has, an optimized expression in a recombinant expression host, the method comprising: creating a library of non-stochastically generated progeny polynucleotides from a parental polynucleotide set; whereby optimization can thus be achieved using one or more of the directed evolution methods as described herein in any combination, permutation and iterative manner; whereby these directed evolution methods include the introduction of mutations by non-stochastic methods, including by "gene site saturation mutagenesis" as described herein; and whereby these directed evolution methods also include the introduction mutations by non-stochastic polynucleotide reassembly methods as described herein; including by synthetic ligation polynucleotide reassembly as described herein.

59. A method for obtaining an immunomodulatory polynucleotide that has an optimized expression in a recombinant expression host, the method comprising: screening a library of non-stochastically generated progeny polynucleotides to identify an optimized non-stochastically generated progeny polynucleotide that has an optimized expression in a recombinant expression host when compared to the expression of a parental polynucleotide from which the library was created.

60. A method for obtaining an immunomodulatory polynucleotide that has an optimized expression in a recombinant expression host, the method comprising: a) creating a library of non-stochastically generated progeny polynucleotides from a parental polynucleotide set; and b) screening a library of non-stochastically generated progeny polynucleotides to identify an optimized non-stochastically generated

expression host when compared to the expression of a parental polynucleotide from which the library was created; whereby optimization can thus be achieved using one or more of the directed evolution methods as described herein in any combination, permutation, and iterative manner; whereby these directed evolution methods include the introduction of point mutations by non-stochastic methods, including by "gene site saturation mutagenesis" as described herein; and whereby these directed evolution methods also include the introduction mutations by non-stochastic polynucleotide reassembly methods as described herein; including by synthetic ligation polynucleotide reassembly as described herein.

61. The method of any of claims 58-60, wherein the recombinant expression host is a prokaryote.

62. The method of any of claims 58-60, wherein the recombinant expression host is a eukaryote.

63. The method of claim 62, wherein the recombinant expression host is a plant.

64. The method of any of claims 63, wherein the recombinant expression host is a monocot.

65. The method of any of claims 63, wherein the recombinant expression host is a dicot.

66. The method of any of claims 1-6, 52, or 58-60, wherein creating a library of non-stochastically generated progeny polynucleotides from a parental polynucleotide set is comprised of subjecting the parental polynucleotide set to "gene site saturation mutagenesis" as described herein.

67. The method of any of claims 1-6, 52, or 58-60, wherein creating a library of non-stochastically generated progeny polynucleotides from a parental polynucleotide set is comprised of subjecting the parental polynucleotide set to "synthetic ligation polynucleotide reassembly" as described herein.

68. The method of any of claims 1-6, 52, or 58-60, wherein creating a library of non-stochastically generated progeny polynucleotides from a parental polynucleotide set is comprised of subjecting the parental polynucleotide set to both "gene site saturation mutagenesis" as described herein, and to "synthetic ligation polynucleotide reassembly" as described herein.

L5 ANSWER 32 OF 112 USPTAFULL on STN

2003:282611 Human cDNAs and proteins and uses thereof.

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US 2003198954 A1 20031023

APPLICATION: US 2001-1142 A1 20011114 (10)

PRIORITY: WO 2001-IB1715 20010806

US 2001-305456P 20010713 (60)

US 2001-302277P 20010629 (60)

US 2001-298698P 20010615 (60)

US 2001-293574P 20010525 (60)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention concerns GENSET polynucleotides and polypeptides. Such GENSET products may be used as reagents in forensic analyses, as chromosome markers, as tissue/cell/organelle-specific markers, in the production of expression vectors. In addition, they may be used in screening and diagnosis assays for abnormal GENSET expression and/or biological activity and for screening compounds that may be used in the treatment of GENSET-related disorders.

CLM What is claimed is:

1. An isolated polynucleotide, comprising a nucleic acid sequence selected from the group consisting of: a) a polynucleotide of SEQ ID NO:77, or of a human cDNA of deposited clone 105-076-4-0-H1-F, encoding at least any single integer from 6 to 500 amino acids of SEQ ID NO:78; b) a polynucleotide of SEQ ID NO:77, or of a human cDNA of deposited clone 105-076-4-0-H1-F, encoding the signal peptide sequence of SEQ ID NO:78; c) a polynucleotide of SEQ ID NO:77, or of a human cDNA of deposited clone 105-076-4-0-H1-F, encoding a mature polypeptide sequence of SEQ ID NO:78; d) a polynucleotide of SEQ ID NO:77, or of a human cDNA of deposited clone 105-076-4-0-H1-F, encoding a full length polypeptide sequence of SEQ ID NO:78; e) a polynucleotide of SEQ ID NO:77, or of a human cDNA of deposited clone 105-076-4-0-H1-F, encoding a polypeptide sequence of a biologically active fragment of SEQ ID

any single integer from 6 to 500 amino acids of SEQ ID NO:78 or of a polypeptide encoded by a human cDNA of deposited clone 105-076-4-0-H1-F; g) a polynucleotide encoding a polypeptide sequence of a signal peptide of SEQ ID NO:78 or of a signal peptide encoded by a human cDNA of deposited clone 105-076-4-0-H1-F; h) a polynucleotide encoding a polypeptide sequence of a mature polypeptide of SEQ ID NO:78 or of a mature polypeptide encoded by a human cDNA of deposited clone 105-076-4-0-H1-F; i) a polynucleotide encoding a polypeptide sequence of a full length polypeptide of SEQ ID NO:78 or of a mature polypeptide encoded by a human cDNA of deposited clone 105-076-4-0-H1-F; j) a polynucleotide encoding a polypeptide sequence of a biologically active polypeptide of SEQ ID NO:78, or of a biologically active polypeptide encoded by a human cDNA of deposited clone 105-076-4-0-H1-F; k) a polynucleotide of any one of a) through j) further comprising an expression vector; l) a host cell recombinant for a polynucleotide of a) through k) above; m) a non-human transgenic animal comprising the host cell of k); and n) a polynucleotide of a) through j) further comprising a physiologically acceptable carrier.

2. A polypeptide comprising an amino acid sequence selected from the group consisting of: a) any single integer from 6 to 500 amino acids of SEQ ID NO:78 or of a polypeptide encoded by a human cDNA of deposited clone 105-076-4-0-H1-F; b) a signal peptide sequence of SEQ ID NO:78 or encoded by a human cDNA of deposited clone 105-076-4-0-H1-F; c) a mature polypeptide sequence of SEQ ID NO:78 or encoded by a human cDNA of deposited clone 105-076-4-0-H1-F; d) a full length polypeptide sequence of SEQ ID NO:78 or encoded by a human cDNA of deposited clone 105-076-4-0-H1-F; and e) a polypeptide of a) through d) further comprising a physiologically acceptable carrier.

3. A method of making a polypeptide, said method comprising: a) providing a population of host cells comprising the polynucleotide of claim 1; b) culturing said population of host cells under conditions conducive to the production of a polypeptide of claim 2 within said host cells; and c) purifying said polypeptide from said population of host cells.

4. A method of making a polypeptide, said method comprising: a) providing a population of cells comprising a polynucleotide encoding the polypeptide of claim 2, operably linked to a promoter; b) culturing said population of cells under conditions conducive to the production of said polypeptide within said cells; and c) purifying said polypeptide from said population of cells.

5. An antibody that specifically binds to the polypeptide of claim 2.

6. A method of binding a polypeptide of claim 2 to an antibody of claim 5, comprising contacting said antibody with said polypeptide under conditions in which antibody can specifically bind to said polypeptide.

7. A method of determining whether a BASI2 gene is expressed within a mammal, said method comprising the steps of: a) providing a biological sample from said mammal; b) contacting said biological sample with either of: i) a polynucleotide that hybridizes under stringent conditions to the polynucleotide of claim 1; or ii) a polypeptide that specifically binds to the polypeptide of claim 2; and c) detecting the presence or absence of hybridization between said polynucleotide and an RNA species within said sample, or the presence or absence of binding of said polypeptide to a protein within said sample; wherein a detection of said hybridization or of said binding indicates that said BASI2 gene is expressed within said mammal.

8. The method of claim 7, wherein said polynucleotide is a **primer**, and wherein said hybridization is detected by detecting the presence of an amplification product comprising the sequence of said **primer**.

9. The method of claim 7, wherein said polypeptide is an antibody.

10. A method of determining whether a mammal has an elevated or reduced level of a BASI2 gene expression, said method comprising the steps of: a) providing a biological sample from said mammal; and b) comparing the amount of the polypeptide of claim 2, or of an RNA species encoding said polypeptide, within said biological sample with a level detected in or expected from a control sample; wherein an increased amount of said polypeptide or said RNA species within said biological sample compared to said level detected in or expected from said control sample indicates that said mammal has an elevated level of said BASI2 gene expression, and wherein a decreased amount of said polypeptide or said RNA species within said biological sample compared to said level detected in or expected from said control sample indicates that said mammal has a reduced level of said BASI2 gene expression.

polypeptide, said method comprising: a) contacting the polypeptide of claim 2 with a test compound; and b) determining whether said compound specifically binds to said polypeptide; wherein a detection that said compound specifically binds to said polypeptide indicates that said compound is a candidate modulator of said BASI2 polypeptide.

12. The method of claim 11, further comprising testing the biological activity of said BASI2 polypeptide in the presence of said candidate modulator, wherein an alteration in the biological activity of said BASI2 polypeptide in the presence of said compound in comparison to the activity in the absence of said compound indicates that the compound is a modulator of said BASI2 polypeptide.

13. A method for the production of a pharmaceutical composition comprising a) identifying a modulator of a BASI2 polypeptide using the method of claim 11; and b) combining said modulator with a physiologically acceptable carrier.

L5 ANSWER 33 OF 112 USPATFULL on STN

2003:271002 Methods for rapid detection and identification of bioagents for environmental testing.

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US 2003190605 A1 20031009

APPLICATION: US 2002-326047 A1 20021218 (10)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Method for detecting and identifying unknown bioagents, including bacteria, viruses and the like, by a combination of nucleic acid amplification and molecular weight determination using primers which hybridize to conserved sequence regions of nucleic acids derived from a bioagent and which bracket variable sequence regions that uniquely identify the bioagent. The result is a "base composition signature" (BCS) which is then matched against a database of base composition signatures, by which the bioagent is identified.

CLM What is claimed is:

1. A method of identifying an unknown bioagent in an environmental sample comprising: a) contacting nucleic acid from said bioagent in said environmental sample with at least one pair of **oligonucleotide primers** which hybridize to sequences of said nucleic acid, wherein said sequences flank a variable nucleic acid sequence of the bioagent; b) amplifying said variable nucleic acid sequence to produce an amplification product; c) determining the molecular mass of said amplification product; and d) comparing said molecular mass to one or more molecular masses of amplification products obtained by performing steps a)-c) on a plurality of known organisms, wherein a match identifies said unknown bioagent in said environmental sample.

2. The method of claim 1 wherein said sequences to which said at least one pair of **oligonucleotide primers** hybridize are highly conserved.

3. The method of claim 1 wherein said amplifying step comprises **polymerase chain reaction**.

4. The method of claim 1 wherein said amplifying step comprises ligase chain reaction or strand displacement amplification.

5. The method of claim 1 wherein said bioagent is a bacterium, virus, parasite, fungi, cell or spore.

6. The method of claim 1 wherein said nucleic acid is ribosomal RNA.

7. The method of claim 1 wherein said nucleic acid encodes RNase P or an RNA-dependent RNA polymerase.

8. The method of claim 1 wherein said amplification product is ionized prior to molecular mass determination.

9. The method of claim 1 further comprising the step of isolating nucleic acid from said bioagent prior to contacting said nucleic acid with said at least one pair of **oligonucleotide primers**.

10. The method of claim 1 further comprising the step of performing steps a)-d) using a different **oligonucleotide primer** pair and comparing the results to one or more molecular mass amplification products obtained by performing steps a)-c) on a different plurality of known organisms from those in step d).

contained in a database of molecular masses.

12. The method of claim 1 wherein said amplification product is ionized by electrospray ionization, matrix-assisted laser desorption or fast atom bombardment.

13. The method of claim 1 wherein said molecular mass is determined by mass spectrometry.

14. The method of claim 11 wherein said mass spectrometry is selected from the group consisting of Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS), ion trap, quadrupole, magnetic sector, time of flight (TOF), Q-TOF and triple quadrupole.

15. The method of claim 1 further comprising performing step b) in the presence of an analog of adenine, thymidine, guanosine or cytidine having a different molecular weight than adenosine, thymidine, guanosine or cytidine.

16. The method of claim 1 wherein said **oligonucleotide primer** comprises a base analog at positions 1 and 2 of each triplet within said **primer**, wherein said base analog binds with increased affinity to its complement compared to the native base.

17. The method of claim 16 wherein said **primer** comprises a universal base at position 3 of each triplet within said **primer**.

18. The method of claim 16 wherein said base analog is selected from the group consisting of 2,6-diaminopurine, propyne T, propyne G, phenoxazines and G-clamp.

19. The method of claim 16 wherein said universal base is selected from the group consisting of inosine, guanidine uridine, 5-nitroindole, 3-nitropyrrole, dP, dK, and 1-(2-deoxy- β -D-ribofuranosyl)-imidazole-4-carboxamide.

20. The method of claim 1 wherein said environmental sample is a water sample, air sample, or land sample.

21. The method of claim 20 wherein said water sample is obtained from a lake, river, ocean, stream, water treatment system, rainwater, groundwater, water table, reservoir, well, or bottled water.

22. The method of claim 20 wherein said air sample is obtained from a ventilation system, airplane cabin, school, hospital, or mass transit location.

23. The method of claim 22 wherein said mass transit location is a subway, train station, or airport.

24. A method of identifying an unknown bioagent in an environmental sample comprising: a) contacting nucleic acid from said bioagent in an environmental sample with at least one pair of **oligonucleotide primers** which hybridize to sequences of said nucleic acid, wherein said sequences flank a variable nucleic acid sequence; b) amplifying said variable nucleic acid sequence to produce an amplification product; c) determining the base composition of said amplification product; and d) comparing said base composition to one or more base compositions of amplification products obtained by performing steps a)-c) on a plurality of known organisms, wherein a match identifies said unknown bioagent in said environmental sample.

25. The method of claim 24 wherein said sequences to which said at least one pair of **oligonucleotide primers** hybridize are highly conserved.

26. The method of claim 24 wherein said amplifying step comprises **polymerase chain reaction**.

27. The method of claim 24 wherein said amplifying step comprises ligase chain reaction or strand displacement amplification.

28. The method of claim 24 wherein said bioagent is a bacterium, virus, fungi, parasite, cell or spore.

29. The method of claim 24 wherein said nucleic acid is ribosomal RNA.

30. The method of claim 24 wherein said nucleic acid encodes RNase P or an RNA-dependent RNA polymerase.

31. The method of claim 24 wherein said amplification product is ionized prior to base composition determination.

nucleic acid from said bioagent prior to contacting said nucleic acid with said at least one pair of **oligonucleotide primers**.

33. The method of claim 24 further comprising the step of performing steps a)-d) using a different **oligonucleotide primer** pair and comparing the results to one or more base compositions of amplification product obtained by performing steps a)-c) on a different plurality of known organisms from those in step d).

34. The method of claim 24 wherein said one or more base composition signatures are contained in a database of base composition signatures.

35. The method of claim 24 wherein said amplification product is ionized by electrospray ionization, matrix-assisted laser desorption or fast atom bombardment.

36. The method of claim 24 wherein said base composition signature is determined by mass spectrometry.

37. The method of claim 36 wherein said mass spectrometry is selected from the group consisting of Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS), ion trap, quadrupole, magnetic sector, time of flight (TOF), q-TOF and triple quadrupole.

38. The method of claim 24 further comprising performing step b) in the presence of an analog of adenine, thymidine, guanosine or cytidine having a different molecular weight than adenosine, thymidine, guanosine or cytidine.

39. The method of claim 24 wherein said **oligonucleotide primer** comprises a base analog at positions 1 and 2 of each triplet within said **primer**, wherein said base analog binds with increased affinity to its complement compared to the native base.

40. The method of claim 39 wherein said **primer** comprises a universal base at position 3 of each triplet within said **primer**.

41. The method of claim 39 wherein said base analog is selected from the group consisting of 2,6-diaminopurine, propyne T, propyne G, phenoxazines and G-clamp.

42. The method of claim 39 wherein said universal base is selected from the group consisting of inosine, guanidine uridine, 5-nitroindole, 3-nitropyrrole, dP, dK, and 1-(2-deoxy- β -D-ribofuranosyl)-imidazole-4-carboxamide.

43. The method of claim 24 wherein said environmental sample is a water sample, air sample, or land sample.

44. The method of claim 43 wherein said water sample is obtained from a lake, river, ocean, stream, water treatment system, rainwater, groundwater, water table, reservoir, well, or bottled water.

45. The method of claim 43 wherein said air sample is obtained from a ventilation system, airplane cabin, school, hospital, or mass transit location.

46. The method of claim 45 wherein said mass transit location is a subway, train station, or airport.

47. A method of determining the absence of a bioagent in an environmental sample comprising: a) contacting said environmental sample suspected of containing nucleic acid encoding said bioagent with at least one pair of **oligonucleotide primers** which are capable of hybridizing to sequences of said nucleic acid, wherein said sequences flank a variable nucleic acid sequence of said bioagent; b) treating said variable nucleic acid sequence under amplification conditions capable of producing an amplification product of said variable nucleic acid sequence; c) performing spectroscopy to determine the molecular mass or base composition of all amplification products; and d) comparing said molecular mass to one or more molecular masses of amplification products or said base composition to one or more base compositions of amplification products obtained by performing steps a)-c) on a plurality of known organisms, wherein the lack of a match indicates that said bioagent is absent from said environmental sample.

48. The method of claim 47 further comprising determining the presence of a positive control.

49. The method of claim 48 wherein said positive control is a known bioagent or residual **primer** signal.

2003:250911 Method for rapid detection and identification of bioagents.

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US 2003175697 A1 20030918

APPLICATION: US 2002-319342 A1 20021213 (10).

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Method for detecting and identifying unknown bioagents, including bacteria, viruses and the like, by a combination of nucleic acid amplification and molecular weight determination using primers which hybridize to conserved sequence regions of nucleic acids derived from a bioagent and which bracket variable sequence regions that uniquely identify the bioagent. The result is a "base composition signature" (BCS) which is then matched against a database of base composition signatures, by which the bioagent is identified.

CLM What is claimed is:

1. A method of identifying an unknown bioagent comprising: (a) contacting nucleic acid from said bioagent with at least one pair of **oligonucleotide primers** which hybridize to sequences of said nucleic acid, wherein said sequences flank a variable nucleic acid sequence of the bioagent; (b) amplifying said variable nucleic acid sequence to produce an amplification product; (c) determining the molecular mass of said amplification product; and (d) comparing said molecular mass to one or more molecular masses of amplification products obtained by performing steps (a)-(c) on a plurality of known organisms, wherein a match identifies said unknown bioagent.
2. The method of claim 1, wherein said sequences to which said at least one pair of **oligonucleotide primers** hybridize are highly conserved.
3. The method of claim 1, wherein said amplifying step comprises **polymerase chain reaction**.
4. The method of claim 1, wherein said amplifying step comprises ligase chain reaction or strand displacement amplification.
5. The method of claim 1, wherein said bioagent is a bacterium, virus, cell or spore.
6. The method of claim 1, wherein said nucleic acid is ribosomal RNA.
7. The method of claim 1, wherein said nucleic acid encodes RNase P or an RNA-dependent RNA polymerase.
8. The method of claim 1, wherein said amplification product is ionized prior to molecular mass determination.
9. The method of claim 1, further comprising the step of isolating nucleic acid from said bioagent prior to contacting said nucleic acid with said at least one pair of **oligonucleotide primers**.
10. The method of claim 1, further comprising the step of performing steps (a)-(d) using a different **oligonucleotide primer** pair and comparing the results to one or more molecular mass amplification product obtained by performing steps (a)-(c) on a different plurality of known organisms from those in step (d).
11. The method of claim 1, wherein said one or more molecular masses are contained in a database of molecular masses.
12. The method of claim 1, wherein said amplification product is ionized by electrospray ionization, matrix-assisted laser desorption or fast atom bombardment.
13. The method of claim 1, wherein said molecular mass is determined by mass spectrometry.
14. The method of claim 11, wherein said mass spectrometry is selected from the group consisting of Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS), ion trap, quadrupole, magnetic sector, time of flight (TOF), Q-TOF and triple quadrupole.
15. The method of claim 1, further comprising performing step (b) in the presence of an analog of adenine, thymidine, guanosine or cytidine having a different molecular weight than adenosine, thymidine, guanosine or cytidine.
16. The method of claim 1, wherein said **oligonucleotide primer**

primer, wherein said base analog binds with increased affinity to its complement compared to the native base.

17. The method of claim 16, wherein said **primer** comprises a universal base at position 3 of each triplet within said **primer**.

18. The method of claim 16, wherein said base analog is selected from the group consisting of 2,6-diaminopurine, propyne T, propyne G, phenoxazines and G-clamp.

19. The method of claim 16, wherein said universal base is selected from the group consisting of inosine, guanidine uridine, 5-nitroindole, 3-nitropyrrole, dP, dK, and 1-(2-deoxy- β -D-ribofuranosyl)-imidazole-4-carboxamide.

20. A method of identifying an unknown bioagent comprising: contacting nucleic acid from said bioagent with at least one pair of **oligonucleotide primers** which hybridize to sequences of said nucleic acid, wherein said sequences flank a variable nucleic acid sequence; amplifying said variable nucleic acid sequence to produce an amplification product; determining the base composition of said amplification product; and comparing said base composition to one or more base compositions of amplification products obtained by performing steps (a)-(c) on a plurality of known organisms, wherein a match identifies said unknown bioagent.

21. The method of claim 20, wherein said sequences to which said at least one pair of **oligonucleotide primers** hybridize are highly conserved.

22. The method of claim 20, wherein said amplifying step comprises **polymerase chain reaction**.

23. The method of claim 20, wherein said amplifying step comprises ligase chain reaction or strand displacement amplification.

24. The method of claim 20, wherein said bioagent is a bacterium, virus, cell or spore.

25. The method of claim 20, wherein said nucleic acid is ribosomal RNA.

26. The method of claim 20, wherein said nucleic acid encodes RNase P or an RNA-dependent RNA polymerase.

27. The method of claim 20, wherein said amplification product is ionized prior to base composition determination.

28. The method of claim 20, further comprising the step of isolating nucleic acid from said bioagent prior to contacting said nucleic acid with said at least one pair of **oligonucleotide primers**.

29. The method of claim 20, further comprising the step of performing steps (a)-(d) using a different **oligonucleotide primer** pair and comparing the results to one or more base compositions of amplification product obtained by performing steps (a)-(c) on a different plurality of known organisms from those in step (d).

30. The method of claim 20, wherein said one or more base composition signatures are contained in a database of base composition signatures.

31. The method of claim 20, wherein said amplification product is ionized by electrospray ionization, matrix-assisted laser desorption or fast atom bombardment.

32. The method of claim 20, wherein said base composition signature is determined by mass spectrometry.

33. The method of claim 32, wherein said mass spectrometry is selected from the group consisting of Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS), ion trap, quadrupole, magnetic sector, time of flight (TOF), q-TOF and triple quadrupole.

34. The method of claim 20, further comprising performing step (b) in the presence of an analog of adenine, thymidine, guanosine or cytidine having a different molecular weight than adenosine, thymidine, guanosine or cytidine.

35. The method of claim 20, wherein said **oligonucleotide primer** comprises a base analog at positions 1 and 2 of each triplet within said **primer**, wherein said base analog binds with increased affinity to its complement compared to the native base.

base at position 3 of each triplet within said **primer**.

37. The method of claim 35, wherein said base analog is selected from the group consisting of 2,6-diaminopurine, propyne T, propyne G, phenoxazines and G-clamp.

38. The method of claim 36, wherein said universal base is selected from the group consisting of inosine, guanidine uridine, 5-nitroindole, 3-nitropyrrole, dP, dK, and 1-(2-deoxy- β -D-ribofuranosyl)-imidazole-4-carboxamide.

39. A method for detecting a single nucleotide polymorphism in an individual, comprising the steps of: isolating nucleic acid from said individual; contacting said nucleic acid with **oligonucleotide primers** which hybridize to regions of said nucleic acid which flank a region comprising said potential polymorphism; amplifying said region to produce an amplification product; determining the molecular mass of said amplification product; comparing said molecular mass to the molecular mass of said region in an individual known to have said polymorphism, wherein if said molecular masses are the same then said individual has said polymorphism.

40. The method of claim 39, wherein said polymorphism is associated with a disease.

41. The method of claim 39, wherein said polymorphism is a blood group antigen.

42. The method of claim 39, wherein said amplification step is the **polymerase chain reaction**.

43. The method of claim 39, wherein said amplification step is ligase chain reaction or strand displacement amplification.

44. The method of claim 39, wherein said amplification product is ionized prior to mass determination.

45. The method of claim 39, wherein said amplification product is ionized by electrospray ionization, matrix-assisted laser desorption or fast atom bombardment.

46. The method of claim 39, wherein said **primers** hybridize to conserved sequences.

47. The method of claim 39, wherein said molecular mass is determined by mass spectrometry.

48. The method of claim 47, wherein said mass spectrometry is selected from the group consisting of Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS), ion trap, quadrupole, magnetic sector, time of flight (TOF), Q-TOF and triple quadrupole.

L5 ANSWER 35 OF 112 USPATFULL on STN

2003:250910 Method for rapid detection and identification of bioagents.

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US 2003175696 A1 20030918

APPLICATION: US 2002-319290 A1 20021213 (10)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Method for detecting and identifying unknown bioagents, including bacteria, viruses and the like, by a combination of nucleic acid amplification and molecular weight determination using primers which hybridize to conserved sequence regions of nucleic acids derived from a bioagent and which bracket variable sequence regions that uniquely identify the bioagent. The result is a "base composition signature" (BCS) which is then matched against a database of base composition signatures, by which the bioagent is identified.

CLM What is claimed is:

1. A method of identifying an unknown bioagent comprising: (a) contacting nucleic acid from said bioagent with at least one pair of **oligonucleotide primers** which hybridize to sequences of said nucleic acid, wherein said sequences flank a variable nucleic acid sequence of the bioagent; (b) amplifying said variable nucleic acid sequence to produce an amplification product; (c) determining the molecular mass of said amplification product; and (d) comparing said molecular mass to one or more molecular masses of amplification products obtained by performing steps (a)-(c) on a plurality of known organisms, wherein a

2. The method of claim 1, wherein said sequences to which said at least one pair of **oligonucleotide primers** hybridize are highly conserved.
3. The method of claim 1, wherein said amplifying step comprises **polymerase chain reaction**.
4. The method of claim 1, wherein said amplifying step comprises ligase chain reaction or strand displacement amplification.
5. The method of claim 1, wherein said bioagent is a bacterium, virus, cell or spore.
6. The method of claim 1, wherein said nucleic acid is ribosomal RNA.
7. The method of claim 1, wherein said nucleic acid encodes RNase P or an RNA-dependent RNA polymerase.
8. The method of claim 1, wherein said amplification product is ionized prior to molecular mass determination.
9. The method of claim 1, further comprising the step of isolating nucleic acid from said bioagent prior to contacting said nucleic acid with said at least one pair of **oligonucleotide primers**.
10. The method of claim 1, further comprising the step of performing steps (a)-(d) using a different **oligonucleotide primer** pair and comparing the results to one or more molecular mass amplification product obtained by performing steps (a)-(c) on a different plurality of known organisms from those in step (d).
11. The method of claim 1, wherein said one or more molecular masses are contained in a database of molecular masses.
12. The method of claim 1, wherein said amplification product is ionized by electrospray ionization, matrix-assisted laser desorption or fast atom bombardment.
13. The method of claim 1, wherein said molecular mass is determined by mass spectrometry.
14. The method of claim 11, wherein said mass spectrometry is selected from the group consisting of Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS), ion trap, quadrupole, magnetic sector, time of flight (TOF), Q-TOF and triple quadrupole.
15. The method of claim 1, further comprising performing step (b) in the presence of an analog of adenine, thymidine, guanosine or cytidine having a different molecular weight than adenosine, thymidine, guanosine or cytidine.
16. The method of claim 1, wherein said **oligonucleotide primer** comprises a base analog at positions 1 and 2 of each triplet within said **primer**, wherein said base analog binds with increased affinity to its complement compared to the native base.
17. The method of claim 16, wherein said **primer** comprises a universal base at position 3 of each triplet within said **primer**.
18. The method of claim 16, wherein said base analog is selected from the group consisting of 2,6-diaminopurine, propyne T, propyne G, phenoxazines and G-clamp.
19. The method of claim 16, wherein said universal base is selected from the group consisting of inosine, guanidine uridine, 5-nitroindole, 3-nitropyrrole, dP, dK, and 1-(2-deoxy- β -D-ribofuranosyl)-imidazole-4-carboxamide.
20. A method of identifying an unknown bioagent comprising: contacting nucleic acid from said bioagent with at least one pair of **oligonucleotide primers** which hybridize to sequences of said nucleic acid, wherein said sequences flank a variable nucleic acid sequence; amplifying said variable nucleic acid sequence to produce an amplification product; determining the base composition of said amplification product; and comparing said base composition to one or more base compositions of amplification products obtained by performing steps (a)-(c) on a plurality of known organisms, wherein a match identifies said unknown bioagent.
21. The method of claim 20, wherein said sequences to which said at least one pair of **oligonucleotide primers** hybridize are highly conserved.

22. The method of claim 20, wherein said amplifying step comprises **polymerase chain reaction**.
23. The method of claim 20, wherein said amplifying step comprises ligase chain reaction or strand displacement amplification.
24. The method of claim 20, wherein said bioagent is a bacterium, virus, cell or spore.
25. The method of claim 20, wherein said nucleic acid is ribosomal RNA.
26. The method of claim 20, wherein said nucleic acid encodes RNase P or an RNA-dependent RNA polymerase.
27. The method of claim 20, wherein said amplification product is ionized prior to base composition determination.
28. The method of claim 20, further comprising the step of isolating nucleic acid from said bioagent prior to contacting said nucleic acid with said at least one pair of **oligonucleotide primers**.
29. The method of claim 20, further comprising the step of performing steps (a)-(d) using a different **oligonucleotide primer** pair and comparing the results to one or more base compositions of amplification product obtained by performing steps (a)-(c) on a different plurality of known organisms from those in step (d).
30. The method of claim 20, wherein said one or more base composition signatures are contained in a database of base composition signatures.
31. The method of claim 20, wherein said amplification product is ionized by electrospray ionization, matrix-assisted laser desorption or fast atom bombardment.
32. The method of claim 20, wherein said base composition signature is determined by mass spectrometry.
33. The method of claim 32, wherein said mass spectrometry is selected from the group consisting of Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS), ion trap, quadrupole, magnetic sector, time of flight (TOF), q-TOF and triple quadrupole.
34. The method of claim 20, further comprising performing step (b) in the presence of an analog of adenine, thymidine, guanosine or cytidine having a different molecular weight than adenosine, thymidine, guanosine or cytidine.
35. The method of claim 20, wherein said **oligonucleotide primer** comprises a base analog at positions 1 and 2 of each triplet within said **primer**, wherein said base analog binds with increased affinity to its complement compared to the native base.
36. The method of claim 35, wherein said **primer** comprises a universal base at position 3 of each triplet within said **primer**.
37. The method of claim 35, wherein said base analog is selected from the group consisting of 2,6-diaminopurine, propyne T, propyne G, phenoxazines and G-clamp.
38. The method of claim 36, wherein said universal base is selected from the group consisting of inosine, guanidine uridine, 5-nitroindole, 3-nitropyrrrole, dP, dK, and 1-(2-deoxy- β -D-ribofuranosyl)-imidazole-4-carboxamide.
39. A method for detecting a single nucleotide polymorphism in an individual, comprising the steps of: isolating nucleic acid from said individual; contacting said nucleic acid with **oligonucleotide primers** which hybridize to regions of said nucleic acid which flank a region comprising said potential polymorphism; amplifying said region to produce an amplification product; determining the molecular mass of said amplification product; comparing said molecular mass to the molecular mass of said region in an individual known to have said polymorphism, wherein if said molecular masses are the same then said individual has said polymorphism.
40. The method of claim 39, wherein said polymorphism is associated with a disease.
41. The method of claim 39, wherein said polymorphism is a blood group antigen.
42. The method of claim 39, wherein said amplification step is the

43. The method of claim 39, wherein said amplification step is ligase chain reaction or strand displacement amplification.
44. The method of claim 39, wherein said amplification product is ionized prior to mass determination.
45. The method of claim 39, wherein said amplification product is ionized by electrospray ionization, matrix-assisted laser desorption or fast atom bombardment.
46. The method of claim 39, wherein said **primers** hybridize to conserved sequences.
47. The method of claim 39, wherein said molecular mass is determined by mass spectrometry.
48. The method of claim 47, wherein said mass spectrometry is selected from the group consisting of Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS), ion trap, quadrupole, magnetic sector, time of flight (TOF), Q-TOF and triple quadrupole.

L5 ANSWER 36 OF 112 USPTAFULL on STN

2003:250909 Method for rapid detection and identification of bioagents.

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US 2003175695 A1 20030918

APPLICATION: US 2002-318881 A1 20021213 (10)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

- AB Method for detecting and identifying unknown bioagents, including bacteria, viruses and the like, by a combination of nucleic acid amplification and molecular weight determination using primers which hybridize to conserved sequence regions of nucleic acids derived from a bioagent and which bracket variable sequence regions that uniquely identify the bioagent. The result is a "base composition signature" (BCS) which is then matched against a database of base composition signatures, by which the bioagent is identified.
- CLM What is claimed is:
1. A method of identifying an unknown bioagent comprising: (a) contacting nucleic acid from said bioagent with at least one pair of **oligonucleotide primers** which hybridize to sequences of said nucleic acid, wherein said sequences flank a variable nucleic acid sequence of the bioagent; (b) amplifying said variable nucleic acid sequence to produce an amplification product; (c) determining the molecular mass of said amplification product; and (d) comparing said molecular mass to one or more molecular masses of amplification products obtained by performing steps (a)-(c) on a plurality of known organisms, wherein a match identifies said unknown bioagent.
 2. The method of claim 1, wherein said sequences to which said at least one pair of **oligonucleotide primers** hybridize are highly conserved.
 3. The method of claim 1, wherein said amplifying step comprises **polymerase chain reaction**.
 4. The method of claim 1, wherein said amplifying step comprises ligase chain reaction or strand displacement amplification.
 5. The method of claim 1, wherein said bioagent is a bacterium, virus, cell or spore.
 6. The method of claim 1, wherein said nucleic acid is ribosomal RNA.
 7. The method of claim 1, wherein said nucleic acid encodes RNase P or an RNA-dependent RNA polymerase.
 8. The method of claim 1, wherein said amplification product is ionized prior to molecular mass determination.
 9. The method of claim 1, further comprising the step of isolating nucleic acid from said bioagent prior to contacting said nucleic acid with said at least one pair of **oligonucleotide primers**.
 10. The method of claim 1, further comprising the step of performing steps (a)-(d) using a different **oligonucleotide primer** pair and comparing the results to one or more molecular mass amplification product obtained by performing steps (a)-(c) on a different plurality of

11. The method of claim 1, wherein said one or more molecular masses are contained in a database of molecular masses.
12. The method of claim 1, wherein said amplification product is ionized by electrospray ionization, matrix-assisted laser desorption or fast atom bombardment.
13. The method of claim 1, wherein said molecular mass is determined by mass spectrometry.
14. The method of claim 11, wherein said mass spectrometry is selected from the group consisting of Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS), ion trap, quadrupole, magnetic sector, time of flight (TOF), Q-TOF and triple quadrupole.
15. The method of claim 1, further comprising performing step (b) in the presence of an analog of adenine, thymidine, guanosine or cytidine having a different molecular weight than adenosine, thymidine, guanosine or cytidine.
16. The method of claim 1, wherein said **oligonucleotide primer** comprises a base analog at positions 1 and 2 of each triplet within said **primer**, wherein said base analog binds with increased affinity to its complement compared to the native base.
17. The method of claim 16, wherein said **primer** comprises a universal base at position 3 of each triplet within said **primer**.
18. The method of claim 16, wherein said base analog is selected from the group consisting of 2,6-diaminopurine, propyne T, propyne G, phenoxazines and G-clamp.
19. The method of claim 16, wherein said universal base is selected from the group consisting of inosine, guanidine uridine, 5-nitroindole, 3-nitropyrrole, dP, dK, and 1-(2-deoxy- β -D-ribofuranosyl)-imidazole-4-carboxamide.
20. A method of identifying an unknown bioagent comprising: contacting nucleic acid from said bioagent with at least one pair of **oligonucleotide primers** which hybridize to sequences of said nucleic acid, wherein said sequences flank a variable nucleic acid sequence; amplifying said variable nucleic acid sequence to produce an amplification product; determining the base composition of said amplification product; and comparing said base composition to one or more base compositions of amplification products obtained by performing steps (a)-(c) on a plurality of known organisms, wherein a match identifies said unknown bioagent.
21. The method of claim 20, wherein said sequences to which said at least one pair of **oligonucleotide primers** hybridize are highly conserved.
22. The method of claim 20, wherein said amplifying step comprises **polymerase chain reaction**.
23. The method of claim 20, wherein said amplifying step comprises ligase chain reaction or strand displacement amplification.
24. The method of claim 20, wherein said bioagent is a bacterium, virus, cell or spore.
25. The method of claim 20, wherein said nucleic acid is ribosomal RNA.
26. The method of claim 20, wherein said nucleic acid encodes RNase P or an RNA-dependent RNA polymerase.
27. The method of claim 20, wherein said amplification product is ionized prior to base composition determination.
28. The method of claim 20, further comprising the step of isolating nucleic acid from said bioagent prior to contacting said nucleic acid with said at least one pair of **oligonucleotide primers**.
29. The method of claim 20, further comprising the step of performing steps (a)-(d) using a different **oligonucleotide primer** pair and comparing the results to one or more base compositions of amplification product obtained by performing steps (a)-(c) on a different plurality of known organisms from those in step (d).
30. The method of claim 20, wherein said one or more base composition signatures are contained in a database of base composition signatures.

31. The method of claim 20, wherein said amplification product is ionized by electrospray ionization, matrix-assisted laser desorption or fast atom bombardment.
32. The method of claim 20, wherein said base composition signature is determined by mass spectrometry.
33. The method of claim 32, wherein said mass spectrometry is selected from the group consisting of Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS), ion trap, quadrupole, magnetic sector, time of flight (TOF), q-TOF and triple quadrupole.
34. The method of claim 20, further comprising performing step (b) in the presence of an analog of adenine, thymidine, guanosine or cytidine having a different molecular weight than adenosine, thymidine, guanosine or cytidine.
35. The method of claim 20, wherein said **oligonucleotide primer** comprises a base analog at positions 1 and 2 of each triplet within said **primer**, wherein said base analog binds with increased affinity to its complement compared to the native base.
36. The method of claim 35, wherein said **primer** comprises a universal base at position 3 of each triplet within said **primer**.
37. The method of claim 35, wherein said base analog is selected from the group consisting of 2,6-diaminopurine, propyne T, propyne G, phenoxazines and G-clamp.
38. The method of claim 36, wherein said universal base is selected from the group consisting of inosine, guanidine uridine, 5-nitroindole, 3-nitropyrrole, dP, dK, and 1-(2-deoxy- β -D-ribofuranosyl)-imidazole-4-carboxamide.
39. A method for detecting a single nucleotide polymorphism in an individual, comprising the steps of: isolating nucleic acid from said individual; contacting said nucleic acid with **oligonucleotide primers** which hybridize to regions of said nucleic acid which flank a region comprising said potential polymorphism; amplifying said region to produce an amplification product; determining the molecular mass of said amplification product; comparing said molecular mass to the molecular mass of said region in an individual known to have said polymorphism, wherein if said molecular masses are the same then said individual has said polymorphism.
40. The method of claim 39, wherein said polymorphism is associated with a disease.
41. The method of claim 39, wherein said polymorphism is a blood group antigen.
42. The method of claim 39, wherein said amplification step is the **polymerase chain reaction**.
43. The method of claim 39, wherein said amplification step is ligase chain reaction or strand displacement amplification.
44. The method of claim 39, wherein said amplification product is ionized prior to mass determination.
45. The method of claim 39, wherein said amplification product is ionized by electrospray ionization, matrix-assisted laser desorption or fast atom bombardment.
46. The method of claim 39, wherein said **primers** hybridize to conserved sequences.
47. The method of claim 39, wherein said molecular mass is determined by mass spectrometry.
48. The method of claim 47, wherein said mass spectrometry is selected from the group consisting of Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS), ion trap, quadrupole, magnetic sector, time of flight (TOF), Q-TOF and triple quadrupole.

PRIORITY: WO 2001-IB1715 20010806

US 2001-305456P 20010713 (60)

US 2001-302277P 20010629 (60)

US 2001-298698P 20010615 (60)

US 2001-293574P 20010525 (60)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention concerns GENSET polynucleotides and polypeptides. Such GENSET products may be used as reagents in forensic analyses, as chromosome markers, as tissue/cell/organelle-specific markers, in the production of expression vectors. In addition, they may be used in screening and diagnosis assays for abnormal GENSET expression and/or biological activity and for screening compounds that may be used in the treatment of GENSET-related disorders.

CLM What is claimed is:

1. An isolated polynucleotide, comprising a nucleic acid sequence selected from the group consisting of: a) a polynucleotide of SEQ ID NO:103, or of a human cDNA of deposited clone 188-13-1-0-G12-F, encoding at least any single integer from 6 to 500 amino acids of SEQ ID NO:104; b) a polynucleotide of SEQ ID NO:103, or of a human cDNA of deposited clone 188-13-1-0-G12-F, encoding the signal peptide sequence of SEQ ID NO:104; c) a polynucleotide of SEQ ID NO:103, or of a human cDNA of deposited clone 188-13-1-0-G12-F, encoding a mature polypeptide sequence of SEQ ID NO:104; d) a polynucleotide of SEQ ID NO:103, or of a human cDNA of deposited clone 188-13-1-0-G12-F, encoding a full length polypeptide sequence of SEQ ID NO:104; e) a polynucleotide of SEQ ID NO:103, or of a human cDNA of deposited clone 188-13-1-0-G12-F, encoding a polypeptide sequence of a biologically active fragment of SEQ ID NO:104; f) a polynucleotide encoding a polypeptide sequence of at least any single integer from 6 to 500 amino acids of SEQ ID NO:104 or of a polypeptide encoded by a human cDNA of deposited clone 188-13-1-0-G12-F; g) a polynucleotide encoding a polypeptide sequence of a signal peptide of SEQ ID NO:104 or of a signal peptide encoded by a human cDNA of deposited clone 188-13-1-0-G12-F; h) a polynucleotide encoding a polypeptide sequence of a mature polypeptide of SEQ ID NO:104 or of a mature polypeptide encoded by a human cDNA of deposited clone 188-13-1-0-G12-F; i) a polynucleotide encoding a polypeptide sequence of a full length polypeptide of SEQ ID NO:104 or of a mature polypeptide encoded by a human cDNA of deposited clone 188-13-1-0-G12-F; j) a polynucleotide encoding a polypeptide sequence of a biologically active polypeptide of SEQ ID NO:104, or of a biologically active polypeptide encoded by a human cDNA of deposited clone 188-13-1-0-G12-F; k) a polynucleotide of any one of a) through j) further comprising an expression vector; l) a host cell recombinant for a polynucleotide of a) through k) above; m) a non-human transgenic animal comprising the host cell of k); and n) a polynucleotide of a) through j) further comprising a physiologically acceptable carrier.

2. A polypeptide comprising an amino acid sequence selected from the group consisting of: a) any single integer from 6 to 500 amino acids of SEQ ID NO:104 or of a polypeptide encoded by a human cDNA of deposited clone 188-13-1-0-G12-F; b) a signal peptide sequence of SEQ ID NO:104 or encoded by a human cDNA of deposited clone 188-13-1-0-G12-F; c) a mature polypeptide sequence of SEQ ID NO:104 or encoded by a human cDNA of deposited clone 188-13-1-0-G12-F; d) a full length polypeptide sequence of SEQ ID NO:104 or encoded by a human cDNA of deposited clone 188-13-1-0-G12-F; and e) a polypeptide of a) through d) further comprising a physiologically acceptable carrier.

3. A method of making a polypeptide, said method comprising: a) providing a population of host cells comprising the polynucleotide of claim 1; b) culturing said population of host cells under conditions conducive to the production of a polypeptide of claim 2 within said host cells; and c) purifying said polypeptide from said population of host cells.

4. A method of making a polypeptide, said method comprising: a) providing a population of cells comprising a polynucleotide encoding the polypeptide of claim 2, operably linked to a promoter; b) culturing said population of cells under conditions conducive to the production of said polypeptide within said cells; and c) purifying said polypeptide from said population of cells.

5. An antibody that specifically binds to the polypeptide of claim 2.

6. A method of binding a polypeptide of claim 2 to an antibody of claim 5, comprising contacting said antibody with said polypeptide under conditions in which antibody can specifically bind to said polypeptide.

7. A method of determining whether a MOBP-81h gene is expressed within a mammal, said method comprising the steps of: a) providing a biological sample from said mammal; b) contacting said biological sample with

conditions to the polynucleotide of claim 1; or ii) a polypeptide that specifically binds to the polypeptide of claim 2; and c) detecting the presence or absence of hybridization between said polynucleotide and an RNA species within said sample, or the presence or absence of binding of said polypeptide to a protein within said sample; wherein a detection of said hybridization or of said binding indicates that said MOBP-81h gene is expressed within said mammal.

8. The method of claim 7, wherein said polynucleotide is a **primer**, and wherein said hybridization is detected by detecting the presence of an amplification product comprising the sequence of said **primer**.

9. The method of claim 7, wherein said polypeptide is an antibody.

10. A method of determining whether a mammal has an elevated or reduced level of a MOBP-81h gene expression, said method comprising the steps of: a) providing a biological sample from said mammal; and b) comparing the amount of the polypeptide of claim 2, or of an RNA species encoding said polypeptide, within said biological sample with a level detected in or expected from a control sample; wherein an increased amount of said polypeptide or said RNA species within said biological sample compared to said level detected in or expected from said control sample indicates that said mammal has an elevated level of said MOBP-81h gene expression, and wherein a decreased amount of said polypeptide or said RNA species within said biological sample compared to said level detected in or expected from said control sample indicates that said mammal has a reduced level of said MOBP-81h gene expression.

11. A method of identifying a candidate modulator of a MOBP-81h polypeptide, said method comprising: a) contacting the polypeptide of claim 2 with a test compound; and b) determining whether said compound specifically binds to said polypeptide; wherein a detection that said compound specifically binds to said polypeptide indicates that said compound is a candidate modulator of said MOBP-81h polypeptide.

12. The method of claim 11, further comprising testing the biological activity of said MOBP-81h polypeptide in the presence of said candidate modulator, wherein an alteration in the biological activity of said MOBP-81h polypeptide in the presence of said compound in comparison to the activity in the absence of said compound indicates that the compound is a modulator of said MOBP-81h polypeptide.

13. A method for the production of a pharmaceutical composition comprising a) identifying a modulator of a MOBP-81h polypeptide using the method of claim 11; and b) combining said modulator with a physiologically acceptable carrier.

L5 ANSWER 38 OF 112 USPTAFULL on STN

2003:243864 Adjuvant compositions.

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US 2003170273 A1 20030911

APPLICATION: US 2002-265083 A1 20021003 (10)

PRIORITY: US 2001-326929P 20011003 (60)

US 2002-373547P 20020417 (60)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Adjuvant compositions comprising type 1 interferon inducers, such as double-stranded RNA, in combination with antigen delivery systems and/or immunostimulatory molecules, such as immunostimulatory nucleic acid sequences, for enhancing the immune response of a coadministered antigen, are described.

CLM What is claimed is:

1. A composition comprising: (1) a type 1 interferon inducer; and (2) an antigen delivery system and/or an immunostimulatory molecule, wherein the composition is capable of increasing the immune response to a coadministered antigen as compared to delivery of the antigen and type 1 interferon inducer alone without the antigen delivery system and/or the immunostimulatory molecule.

2. The composition of claim 1, wherein the composition comprises a type 1 interferon inducer, an antigen delivery system and an immunostimulatory molecule.

3. The composition of claim 1, wherein the composition comprises a type 1 interferon inducer and an antigen delivery system.

4. The composition of claim 1, wherein the coadministered antigen is present in the composition.

5. The composition of claim 1, wherein the type 1 interferon inducer is

6. The composition of claim 1, wherein the antigen delivery system comprises a submicron oil-in-water emulsion and/or a microparticle.
7. The composition of claim 1, wherein the immunostimulatory molecule is an immunostimulatory nucleic acid sequence (ISS).
8. The composition of claim 7, wherein the ISS is a CpG **oligonucleotide**.
9. A composition comprising a dsRNA, a submicron oil-in-water emulsion and a selected antigen, wherein the composition is capable of increasing the immune response to the antigen as compared to delivery of the antigen and dsRNA alone without the submicron oil-in-water emulsion.
10. The composition of claim 9, wherein the antigen is an HCV antigen, an HIV antigen or a meningococcal protein.
11. The composition of claim 10, wherein the antigen is an HCV antigen and the HCV antigen is an E1E2 polypeptide.
12. The composition of claim 10, wherein the antigen is an HIV antigen and the HIV antigen is gp120 or p55gag.
13. The composition of claim 10, wherein the antigen is a meningococcal antigen and the meningococcal antigen is a MenB protein from ORFs 287 and/or 961.
14. The composition of claim 9, wherein the antigen is associated with a microparticle.
15. The composition of claim 14, wherein the antigen is adsorbed to a microparticle comprising poly(D,L-lactide-co-glycolide) (PLG).
16. The composition of claim 9, wherein the dsRNA is associated with a microparticle.
17. The composition of claim 16, wherein the dsRNA is adsorbed to a microparticle comprising poly(D,L-lactide-co-glycolide) (PLG).
18. The composition of claim 9, wherein the dsRNA is viral dsRNA or synthetic dsRNA.
19. The composition of claim 18, wherein the dsRNA is polyriboinosinic-polyribocytidylic acid (poly[rI-rC]), polyriboguanyl-polyribocytidylic acid (poly[rG-rC]) or polyriboadenylic-polyribouridylic acid (poly[rA-rU]).
20. The composition of claim 9, wherein the submicron oil-in-water emulsion comprises: (1) a metabolizable oil, wherein the oil is present in an amount of 0.5% to 20% of the total volume and (2) an emulsifying agent, wherein the emulsifying agent is 0.01% to 2.5% by weight (w/v), and wherein the oil and the emulsifying agent are present in the form of an oil-in-water emulsion having oil droplets substantially all of which are about 100 nm to less than 1 micron in diameter.
21. The composition of claim 20, wherein the emulsifying agent comprises a polyoxyethylene sorbitan mono-, di-, or triester and/or a sorbitan mono-, di-, or triester.
22. A composition comprising: (1) a dsRNA, wherein the dsRNA is polyriboinosinic-polyribocytidylic acid (poly[rI-rC]), polyriboguanyl-polyribocytidylic acid (poly[rG-rC]) or polyriboadenylic-polyribouridylic acid (poly[rA-rU]); (2) a submicron oil-in-water emulsion, wherein the submicron oil-in-water emulsion comprises (a) a metabolizable oil, wherein the oil is present in an amount of 0.5% to 20% of the total volume and (b) an emulsifying agent, wherein the emulsifying agent is 0.01% to 2.5% by weight (w/v), and wherein the oil and the emulsifying agent are present in the form of an oil-in-water emulsion having oil droplets substantially all of which are about 100 nm to less than 1 micron in diameter; and (3) a selected antigen, wherein the selected antigen is an HCV, HIV or a meningococcal antigen, and wherein the composition is capable of increasing the immune response to the antigen as compared to delivery of the antigen alone, without the dsRNA and/or the submicron oil-in-water emulsion.
23. The composition of claim 22, wherein the antigen is adsorbed to a microparticle comprising poly(D,L-lactide-co-glycolide) (PLG).
24. The composition of claim 22, wherein the dsRNA is adsorbed to a microparticle comprising poly(D,L-lactide-co-glycolide) (PLG).
25. The composition of claim 23, wherein the dsRNA is adsorbed is a

26. The composition of claim 22, wherein the oil is present in an amount of 1% to 12% of the total volume and the emulsifying agent is present in an amount of 0.01% to 1% by weight (w/v).

27. The composition of claim 22, wherein the emulsifying agent comprises a polyoxyethylene sorbitan mono-, di-, or triester and/or a sorbitan mono-, di-, or triester.

28. The composition of claim 26, wherein the submicron oil-in-water emulsion comprises 4-5% w/v squalene, 0.25-1.0% w/v polyoxyethylenesorbitan monooleate, and/or 0.25-1.0% sorbitan trioleate.

29. The composition of claim 22, wherein the submicron oil-in-water emulsion consists essentially of 5% by volume of squalene; and one or more emulsifying agents selected from the group consisting of polyoxyethylenesorbitan monooleate and sorbitan trioleate, wherein the total amount of emulsifying agent(s) present is 1% by weight (w/v).

30. The composition of claim 29, wherein the one or more emulsifying agents are polyoxyethylenesorbitan monooleate and sorbitan trioleate and the total amount of polyoxyethylenesorbitan monooleate and sorbitan trioleate present is 1% by weight (w/v).

31. The composition of claim 22, further comprising an immunostimulatory molecule.

32. The composition of claim 31, wherein the immunostimulatory molecule is an immunostimulatory nucleic acid sequence (ISS).

33. The composition of claim 32, wherein the ISS is a CpG **oligonucleotide**.

34. The composition of claim 33, wherein the CpG **oligonucleotide** comprises the sequence 5'-X₁X₂CX₃X₄, where X₁ and X₂ are a sequence selected from the group consisting of GpT, GpG, GpA, ApA, ApT, ApG, CpT, CpA, CpG, TpA, TpT and TpG; and X₃ and X₄ are selected from the group consisting of TpT, CpT, ApT, ApG, CpG, TpC, ApC, CpC, TpA, ApA, GpT, CpA, and TpG, wherein p signifies a phosphate bond.

35. The composition of claim 33, wherein the CpG **oligonucleotide** comprises the sequence GACGTT, GACGTC, GTCGTT or GTCGCT.

36. The composition of claim 35, wherein the CpG motif is 5'-TCCATGACGTTCTGACGTT-3' (SEQ ID NO: 3).

37. The composition of claim 22, wherein the antigen is an HCV antigen and the HCV antigen is an E1E2 polypeptide.

38. The composition of claim 22, wherein the antigen is an HIV antigen and the HIV antigen is gp120 or p55gag.

39. The composition of claim 22, wherein the antigen is a meningococcal antigen and the meningococcal antigen is a MenB protein from ORFs 287 and/or 961.

40. A composition comprising: (1) polyribonucleosinic-polyribocytidylic acid (poly[rI-rC]); (2) a submicron oil-in-water emulsion, wherein the submicron oil-in-water emulsion comprises (a) a metabolizable oil, wherein the oil is present in an amount of 0.5% to 20% of the total volume and (b) an emulsifying agent, wherein the emulsifying agent is 0.01% to 2.5% by weight (w/v), and wherein the oil and the emulsifying agent are present in the form of an oil-in-water emulsion having oil droplets substantially all of which are about 100 nm to less than 1 micron in diameter; (3) a CpG **oligonucleotide**; and (4) an HCV antigen, an HIV antigen or a meningococcal protein, and wherein the composition is capable of increasing the immune response to the antigen as compared to delivery of the antigen alone, without the dsRNA, the submicron oil-in-water emulsion and/or the CpG **oligonucleotide**.

41. The composition of claim 40, wherein the antigen is an HCV antigen and the HCV antigen is an E1E2 polypeptide.

42. The composition of claim 41, wherein the HCV E1E2 polypeptide comprises a sequence of amino acids with at least 80% sequence identity to the contiguous sequence of amino acids depicted at positions 192-809 of FIGS. 1A-1C.

43. The composition of claim 42, wherein the HCV E1E2 polypeptide comprises the sequence of amino acids depicted at positions 192-809 of

44. The composition of claim 40, wherein the antigen is an HIV antigen and the HIV antigen is gp120 or p55gag.,

45. The composition of claim 40, wherein the antigen is a meningococcal antigen and the meningococcal antigen is a MenB protein from ORFs 287 and/or 961.

46. The composition of claim 40, wherein the CpG **oligonucleotide** comprises the sequence 5'-X₁X₂CGX₃X₄, where X₁ and X₂ are a sequence selected from the group consisting of GpT, GpG, GpA, ApA, ApT, ApG, CpT, CpA, CpG, TpA, TpT and TpG; and X₃ and X₄ are selected from the group consisting of TpT, CpT, ApT, ApG, CpG, TpC, ApC, CpC, TpA, ApA, GpT, CpA, and TpG, wherein p signifies a phosphate bond.

47. The composition of claim 40, wherein the CpG **oligonucleotide** comprises the sequence GACGTT, GACGTC, GTCGTT or GTCGCT.

48. The composition of claim 47, wherein the CpG motif is 5'-TCCATGACGTTCTGACGTT-3' (SEQ ID NO: 3).

49. The composition of claim 40, wherein the oil is present in an amount of 1% to 12% of the total volume and the emulsifying agent is present in an amount of 0.01% to 1% by weight (w/v).

50. The composition of claim 40, wherein the emulsifying agent comprises a polyoxyethylene sorbitan mono-, di-, or triester and/or a sorbitan mono-, di-, or triester.

51. The composition of claim 40, wherein the submicron oil-in-water emulsion comprises 4-5% w/v squalene, 0.25-1.0% w/v polyoxyelthylenesorbitan monooleate, and/or 0.25-1.0% sorbitan trioleate.

52. The composition of claim 40, wherein the submicron oil-in-water emulsion consists essentially of 5% by volume of squalene; and one or more emulsifying agents selected from the group consisting of polyoxyelthylenesorbitan monooleate and sorbitan trioleate, wherein the total amount of emulsifying agent(s) present is 1% by weight (w/v).

53. The composition of claim 52, wherein the one or more emulsifying agents are polyoxyclthylenesorbitan monooleate and sorbitan trioleate and the total amount of polyoxyelthylenesorbitan monooleate and sorbitan trioleate present is 1% by weight (w/v).

54. A composition comprising: (1) dsRNA, wherein the dsRNA is polyriboninosinic-polyribocytidylic acid (poly[rI-rC]), polyriboguanylic-polyribocytidylic acid (poly[rG-rC]) or polyriboadenylic-polyribouridylic acid (poly[rA-rU]); and (2) a selected antigen, wherein said selected antigen is an HCV antigen, an HIV antigen or a meningococcal protein, wherein said dsRNA and/or said selected antigen are adsorbed to a microparticle, and further wherein the composition is capable of increasing the immune response to the selected antigen as compared to delivery of the antigen alone without the dsRNA and/or the microparticle.

55. The composition of claim 54, wherein the dsRNA is poly[rI-rC].

56. The composition of claim 55, wherein the microparticle comprises a polymer selected from the group consisting of a poly(α -hydroxy acid), a polyhydroxy butyric acid, a polycaprolactone, a polyorthoester, and a polyanhydride.

57. The composition of claim 56, wherein the microparticle comprises poly(D,L-lactide-co-glycolide) (PLG).

58. The composition of claim 57, wherein the antigen is adsorbed to the microparticle.

59. The composition of claim 57, wherein the dsRNA is adsorbed to the microparticle.

60. The composition of claim 58, wherein the dsRNA is adsorbed to the microparticle.

61. The composition of claim 57, wherein the antigen is an HCV antigen and the HCV antigen is an E1E2 polypeptide.

62. The composition of claim 61, wherein the HCV E1E2 polypeptide comprises a sequence of amino acids with at least 80% sequence identity to the contiguous sequence of amino acids depicted at positions 192-809

63. The composition of claim 62, wherein the HCV E1E2 polypeptide comprises the sequence of amino acids depicted at positions 192-809 of FIGS. 1A-1C.

64. The composition of claim 58, wherein the antigen is an HIV antigen and the HIV antigen is gp120 or p55 gag.

65. The composition of claim 58, wherein the antigen is a meningococcal antigen and the meningococcal antigen is a MenB protein from ORFs 287 and/or 961.

66. A method of stimulating an immune response in a vertebrate subject, said method comprising administering to the subject a therapeutically effective amount of a selected antigen and a composition according to claim 1.

67. The method of claim 66, wherein the antigen is administered prior to administering the composition.

68. The method of claim 66, wherein the antigen is administered subsequent to administering the composition.

69. A method of stimulating an immune response in a vertebrate subject, said method comprising administering to the subject a therapeutically effective amount of a composition according to claim 4.

70. A method of stimulating an immune response in a vertebrate subject, said method comprising administering to the subject a therapeutically effective amount of a composition according to claim 9.

71. A method of stimulating an immune response in a vertebrate subject, said method comprising administering to the subject a therapeutically effective amount of a composition according to claim 22.

72. A method of stimulating an immune response in a vertebrate subject, said method comprising administering to the subject a therapeutically effective amount of a composition according to claim 40.

73. A method of stimulating an immune response in a vertebrate subject, said method comprising administering to the subject a therapeutically effective amount of a composition according to claim 54.

74. A method of making a composition comprising combining a type 1 interferon inducer with an antigen delivery system and/or an immunostimulatory molecule.

75. The method of claim 74, wherein the method further comprises combining an antigen with said type 1 interferon inducer, and said antigen delivery system and/or said immunostimulatory molecule.

L5 ANSWER 39 OF 112 USPTAFULL on STN

2003:237673 Arrays of nucleic acid probes for analyzing biotransformation genes

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US 2003165830 A1 20030904

APPLICATION: US 2001-798260 A1 20010301 (9)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention provides arrays of immobilized probes, and methods employing the arrays, for detecting mutations in the biotransformation genes, such as cytochromes P450. For example, one such array comprises four probe sets. A first probe set comprises a plurality of probes, each probe comprising a segment of at least three nucleotides exactly complementary to a subsequence of a reference sequence from a biotransformation gene, the segment including at least one interrogation position complementary to a corresponding nucleotide in the reference sequence. Second, third and fourth probe sets each comprise a corresponding probe for each probe in the first probe set. The probes in the second, third and fourth probe sets are identical to a sequence comprising the corresponding probe from the first probe set or a subsequence of at least three nucleotides thereof that includes the at

interrogation position is occupied by a different nucleotide in each of the four corresponding probes from the four probe sets.

What is claimed is:

1. An array of **oligonucleotide** probes immobilized on a solid support, the array comprising at least two sets of **oligonucleotide** probes, (1) a first probe set comprising a plurality of probes, each probe comprising a segment of at least three nucleotides exactly complementary to a subsequence of a reference sequence, the segment including at least one interrogation position complementary to a corresponding nucleotide in the reference sequence, (2) a second probe set comprising a corresponding probe for each probe in the first probe set, the corresponding probe in the second probe set being identical to a sequence comprising the corresponding probe from the first probe set or a subsequence of at least three nucleotides thereof that includes the at least one interrogation position, except that the at least one interrogation position is occupied by a different nucleotide in each of the two corresponding probes from the first and second probe sets; wherein the probes in the first probe set have at least three interrogation positions respectively corresponding to each of three contiguous nucleotides in the reference sequence; provided that the array does not contain a complete set of probes of a given length; wherein the reference sequence is from a biotransformation gene.
2. An array of **oligonucleotide** probes immobilized on a solid support, the array comprising at least four sets of **oligonucleotide** probes, (1) a first probe set comprising a plurality of probes, each probe comprising a segment of at least three nucleotides exactly complementary to a subsequence of a reference sequence, the segment including at least one interrogation position complementary to a corresponding nucleotide in the reference sequence, (2) second, third and fourth probe sets, each comprising a corresponding probe for each probe in the first probe set, the probes in the second, third and fourth probe sets being identical to a sequence comprising the corresponding probe from the first probe set or a subsequence of at least three nucleotides thereof that includes the at least one interrogation position, except that the at least one interrogation position is occupied by a different nucleotide in each of the four corresponding probes from the four probe sets; provided the array lacks a complete set of probes of a given length; wherein the reference sequence is from a biotransformation gene.
3. The array of claim 1 wherein the reference sequence is from a gene encoding an enzyme selected from the group consisting of a cytochrome P450, N-acetyl transferase II, glucose 6-phosphate dehydrogenase, pseudocholinesterase, catechol-O-methyl transferase, and dihydropyridine dehydrogenase.
4. The array of claim 2, wherein the reference sequence is from a gene encoding an enzyme selected from the group consisting of a cytochrome P450, N-acetyl transferase II, glucose 6-phosphate dehydrogenase, pseudocholinesterase, catechol-O-methyl transferase, and dihydropyridine dehydrogenase.
5. The array of claim 4, wherein the enzyme is P450 2D6 or P450 2C19.
6. The array of claim 2, wherein the reference sequence includes a site of a mutation and a site of a silent polymorphism.
7. The array of claim 6, wherein the silent polymorphism is in an intron or flanking region of a gene.
8. The array of claim 2, wherein the first probe set has at least 3 interrogation positions respectively corresponding to each of 3 contiguous nucleotides in the reference sequence.
9. The **oligonucleotide** array of claim 2, wherein the array has between 100 and 100,000 probes.
10. The **oligonucleotide** array of claim 2, wherein the probes are linked to the support via a spacer.
11. The **oligonucleotide** array of claim 2, wherein the segment in each probe of the first probe set that is exactly complementary to the subsequence of the reference sequence is 9-21 nucleotides.
12. An array of **oligonucleotide** probes immobilized on a solid support, the array comprising at least one pair of first and second probe groups, each group comprising a first and second sets of **oligonucleotide** probes as defined by claim 1; wherein each probe in the first probe set from the first group is exactly complementary to a subsequence of a first reference sequence and each probe in the first probe set from the second group is exactly complementary to a subsequence from a second

13. The array of claim 12, wherein each group further comprises third and fourth probe sets, each comprising a corresponding probe for each probe in the first probe set, the probes in the second, third and fourth probe sets being identical to a sequence comprising the corresponding probe from the first probe set or a subsequence of at least three nucleotides thereof that includes the interrogation position, except that the interrogation position is occupied by a different nucleotide in each of the four corresponding probes from the four probe sets.

14. The array of claim 12, wherein the first reference sequence includes the site of a mutation in the biotransformation gene, and the second reference sequence includes a site of a silent polymorphism within the biotransformation gene or flanking the biotransformation gene.

15. The array of claim 14, wherein the reference sequence is from a gene encoding an enzyme selected from the group consisting of a cytochrome P450, N-acetyl transferase II, glucose 6-phosphate dehydrogenase, pseudocholinesterase, catechol-O-methyl transferase, and dihydropyridine dehydrogenase.

16. The array of claim 14 that comprises at least forty pairs of first and second probe groups, wherein the probes in the first probe sets from the first groups of the forty pairs are exactly complementary to subsequences from forty respective first reference sequences.

17. A block of **oligonucleotide** probes immobilized on a solid support, comprising: a perfectly matched probe comprising a segment of at least three nucleotides exactly complementary to a subsequence of a reference sequence, the segment having a plurality of interrogation positions respectively corresponding to a plurality of nucleotides in the reference sequence, for each interrogation position, three mismatched probes, each identical to a sequence comprising the perfectly matched probe or a subsequence of at least three nucleotides thereof including the plurality of interrogation positions, except in the interrogation position, which is occupied by a different nucleotide in each of the three mismatched probes and the perfectly matched probe; provided the array lacks a complete set of probes of a given length; wherein the reference sequence is from a biotransformation gene.

18. The array of claim 16, wherein the segment of the perfectly matched probe comprises 3-20 interrogation positions corresponding to 3-20 respective nucleotides in the reference sequence.

19. An array of probes immobilized to a solid support comprising at least two blocks of probes, each block as defined by claim 16, a first block comprising a perfectly matched probe comprising a segment exactly complementary to a subsequence of a first reference sequence and a second block comprising a perfectly matched probe comprising a segment exactly complementary to a subsequence of a second reference sequence.

20. The array of claim 19, wherein the first reference sequence is from a wildtype 2D6 gene and the second reference sequence is from a mutant 2D6 gene.

21. The array of claim 19, comprising at least 10-100 blocks of probes, each comprising a perfectly matched probe comprising a segment exactly complementary to a subsequence of at least 10-100 respective reference sequences.

22. An array of **oligonucleotide** probes immobilized on a solid support, the array comprising at least four probes: a first probe comprising first and second segments, each of at least three nucleotides and exactly complementary to first and second subsequences of a reference sequence, the segments including at least one interrogation position corresponding to a nucleotide in the reference sequence, wherein either (1) the first and second subsequences are noncontiguous, or (2) the first and second subsequences are contiguous and the first and second segments are inverted relative to the complement of the first and second subsequences in the reference sequence; second, third and fourth probes, identical to a sequence comprising the first probe or a subsequence thereof comprising at least three nucleotides from each of the first and second segments, except in the at least one interrogation position, which differs in each of the probes; provided the array lacks a complete set of probes of a given length; wherein the reference sequence is from a biotransformation gene.

23. A method of comparing a target nucleic acid with a reference sequence comprising a predetermined sequence of nucleotides, the method comprising: (a) hybridizing a sample comprising the target nucleic acid to an array of **oligonucleotide** probes immobilized on a solid support, the array comprising: (1) a first probe set comprising a plurality of

exactly complementary to a subsequence of the reference sequence, the segment including at least one interrogation position complementary to a corresponding nucleotide in the reference sequence, wherein the reference sequence is from a biotransformation gene; (2) a second probe set comprising a corresponding probe for each probe in the first probe set, the corresponding probe in the second probe set being identical to a sequence comprising the corresponding probe from the first probe set or a subsequence of at least three nucleotides thereof that includes the at least one interrogation position, except that the at least one interrogation position is occupied by a different nucleotide in each of the two corresponding probes from the first and second probe sets; wherein, the probes in the first probe set have at least three interrogation positions respectively corresponding to each of at least three nucleotides in the reference sequence, and (b) determining which probes, relative to one another, in the first and second probe sets specifically bind to the target nucleic acid, the relative specific binding of corresponding probes in the first and second probe sets indicating whether a nucleotide in the target sequence is the same or different from the corresponding nucleotide in the reference sequence.

24. The method of claim 23, wherein the determining step comprises: (1) comparing the relative specific binding of two corresponding probes from the first and second probe sets; (2) assigning a nucleotide in the target sequence as the complement of the interrogation position of the probe having the greater specific binding; and (3) repeating (1) and (2) until each nucleotide of interest in the target sequence has been assigned.

25. The method of claim 23, wherein the array further comprises third and fourth probe sets, each comprising a corresponding probe for each probe in the first probe set, the probes in the second, third and fourth probe sets being identical to a sequence comprising the corresponding probe from the first probe set or a subsequence of at least three nucleotides thereof that includes the at least one interrogation position, except that the at least one interrogation position is occupied by a different nucleotide in each of the four corresponding probes from the four probe sets; and the determining step comprises determining which probes, relative to one another, in the first, second, third and fourth probe sets specifically bind to the target nucleic acid, the relative specific binding of corresponding probes in the first, second, third and fourth probe sets indicating whether a nucleotide in the target sequence is the same or different from the corresponding nucleotide in the reference sequence.

26. The method of claim 25, wherein: the reference sequence includes a site of a mutation in the biotransformation gene and a silent polymorphism in or flanking the biotransformation gene; the target nucleic acid comprises one or more different alleles of the biotransformation gene; and the relative specific binding of probes having an interrogation position aligned with the silent polymorphism indicates the number of different alleles and the relative specific binding of probes having an interrogation position aligned with the mutation indicates whether the mutation is present in at least one of the alleles.

27. The method of claim 25, wherein the determining comprises: (1) comparing the relative specific binding of four corresponding probes from the first, second, third and fourth probe sets; (2) assigning a nucleotide in the target sequence as the complement of the interrogation position of the probe having the greatest specific binding; (3) repeating (1) and (2) until each nucleotide of interest in the target sequence has been assigned.

28. A method of comparing a target nucleic acid with a reference sequence comprising a predetermined sequence of nucleotides, the method comprising: (a) hybridizing the target nucleic acid to an array of **oligonucleotide** probes immobilized on a solid support, the array comprising: a perfectly matched probe comprising a segment of at least three nucleotides exactly complementary to a subsequence of a reference sequence, the segment having a plurality of interrogation positions respectively corresponding to a plurality of nucleotides in the reference sequence, wherein the reference sequence is from a biotransformation gene; for each interrogation position, three mismatched probes, each identical to a sequence comprising the perfectly matched probe or a subsequence of at least three nucleotides thereof including the plurality of interrogation positions, except in the interrogation position, which is occupied by a different nucleotide in each of the three mismatched probes and the perfectly matched probe; (b) for each interrogation position, (1) comparing the relative specific binding of the three mismatched probes and the perfectly matched probe; (2) assigning a nucleotide in the target sequence as the complement of the interrogation position of the probe having the

29. The method of claim 28, wherein the target sequence has an undetermined substitution relative to the reference sequence, and the method assigns a nucleotide to the substitution.

30. A method of screening a patient for capacity to metabolize a drug, the method comprising: (a) hybridizing a tissue sample from the patient containing a target nucleic acid to an array of **oligonucleotide** probes immobilized on a solid support, the array comprising: (1) a first probe set comprising a plurality of probes, each probe comprising a segment of at least three nucleotides exactly complementary to a subsequence of the reference sequence from a biotransformation gene which metabolizes the drug, the segment including at least one interrogation position complementary to a corresponding nucleotide in the reference sequence, (2) a second probe set comprising a corresponding probe for each probe in the first probe set, the corresponding probe in the second probe set being identical to a sequence comprising the corresponding probe from the first probe set or a subsequence of at least three nucleotides thereof that includes the at least one interrogation position, except that the at least one interrogation position is occupied by a different nucleotide in each of the two corresponding probes from the first and second probe sets; wherein, the probes in the first probe set have at least three interrogation positions respectively corresponding to each of at least three nucleotides in the reference sequence, and (b) determining which probes, relative to one another, in the first and second probe sets specifically to the target nucleic acid, the relative specific binding of corresponding probes in the first and second probe sets indicating whether the target sequence contains a mutation relative to the reference sequence, which, if present, impairs the capacity of the patient to metabolize the drug.

31. A method of conducting a clinical trial on a drug, the method comprising: (a) obtaining a tissue sample containing a target nucleic acid from each of a pool of patients; (b) for each tissue sample, hybridizing the target nucleic acid to an array of **oligonucleotide** probes immobilized on a solid support, the array comprising: (1) a first probe set comprising a plurality of probes, each probe comprising a segment of at least three nucleotides exactly complementary to a subsequence of the reference sequence from a biotransformation gene, the segment including at least one interrogation position complementary to a corresponding nucleotide in the reference sequence, (2) a second probe set comprising a corresponding probe for each probe in the first probe set, the corresponding probe in the second probe set being identical to a sequence comprising the corresponding probe from the first probe set or a subsequence of at least three nucleotides thereof that includes the at least one interrogation position, except that the at least one interrogation position is occupied by a different nucleotide in each of the two corresponding probes from the first and second probe sets; wherein, the probes in the first probe set have at least three interrogation positions respectively corresponding to each of at least three nucleotides in the reference sequence; (c) determining which probes, relative to one another, in the first and second probe sets specifically to the target nucleic acid, the relative specific binding of corresponding probes in the first and second probe sets indicating whether the target sequence contains a mutation relative to the reference sequence selecting a subpool of patients having a target sequence free of the mutation; and (d) administering the drug to the subpool of patients to determine efficacy.

32. The method of claim 31, further comprising combining the drug with a pharmaceutical carrier to form a pharmaceutical composition.

L5 ANSWER 40 OF 112 USPATFULL on STN

2003:237666 Arrays of nucleic acid probes on biological chips.

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US 2003165823 A1 20030904

APPLICATION: US 2000-510378 A1 20000222 (9)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention provides arrays of immobilized probes, and methods employing the arrays, for detecting mutations in the CFTR gene.

CLM What is claimed is:

the array comprising at least two sets of **oligonucleotide** probes, (1) a first probe set comprising a plurality of probes, each probe comprising a segment of at least three nucleotides exactly complementary to a subsequence of a reference sequence, the segment including at least one interrogation position complementary to a corresponding nucleotide in the reference sequence, (2) a second probe set comprising a corresponding probe for each probe in the first probe set, the corresponding probe in the second probe set being identical to a sequence comprising the corresponding probe from the first probe set or a subsequence of at least three nucleotides thereof that includes the at least one interrogation position, except that the at least one interrogation position is occupied by a different nucleotide in each of the two corresponding probes from the first and second probe sets; wherein the probes in the first probe set have at least three interrogation positions respectively corresponding to each of three contiguous nucleotides in the reference sequence; provided that the array does not contain a complete set of probes of a given length; wherein the reference sequence is from a CFTR gene.

2. An array of **oligonucleotide** probes immobilized on a solid support, the array comprising at least four sets of **oligonucleotide** probes, (1) a first probe set comprising a plurality of probes, each probe comprising a segment of at least three nucleotides exactly complementary to a subsequence of a reference sequence, the segment including at least one interrogation position complementary to a corresponding nucleotide in the reference sequence, (2) second, third and fourth probe sets, each comprising a corresponding probe for each probe in the first probe set, the probes in the second, third and fourth probe sets being identical to a sequence comprising the corresponding probe from the first probe set or a subsequence of at least three nucleotides thereof that includes the at least one interrogation position, except that the at least one interrogation position is occupied by a different nucleotide in each of the four corresponding probes from the four probe sets; provided the array lacks a complete set of probes of a given length; wherein the reference sequence is from a CFTR gene.

3. The **oligonucleotide** array of claim 2, further comprising a fifth probe set comprising a corresponding probe for each probe in the first probe set, the corresponding probe from the fifth probe set being identical to a sequence comprising the corresponding probe from the first probe set or a subsequence of at least three nucleotides thereof that includes the at least one interrogation position, except that the at least one interrogation position is deleted in the corresponding probe from the fifth probe set.

4. The **oligonucleotide** array of claim 2, further comprising a sixth probe set comprising a corresponding probe for each probe in the first probe set, the corresponding probe from the sixth probe set being identical to a sequence comprising the corresponding probe from the first probe set or a subsequence of at least three nucleotides thereof that includes the at least one interrogation position, except that an additional nucleotide is inserted adjacent to the at least one interrogation position in the corresponding probe from the first probe set.

5. The array of claim 2, wherein the first probe set has at least three interrogation positions respectively corresponding to each of three contiguous nucleotides in the reference sequence.

6. The array of claim 2, wherein the first probe set has at least 50 interrogation positions respectively corresponding to each of 50 contiguous nucleotides in the reference sequence.

7. The **oligonucleotide** array of claim 2, wherein the array has between 100 and 100,000 probes.

8. The **oligonucleotide** array of claim 2, wherein the probes are linked to the support via a spacer.

9. The **oligonucleotide** array of claim 2, wherein the segment in each probe of the first probe set that is exactly complementary to the subsequence of the reference sequence is 9-21 nucleotides.

10. The **oligonucleotide** array of claim 2, wherein each probe of the first probe set consists of the segment that is exactly complementary to the subsequence of the reference sequence.

11. The **oligonucleotide** array of claim 2, wherein the probes in the second, third and fourth probe sets are identical to the corresponding probe from the first probe set except that the at least one interrogation position is occupied by a different nucleotide in each of the four corresponding probes from the four probe sets.

12. An array of **oligonucleotide** probes immobilized on a solid support, the array comprising at least one pair of first and second probe groups, each group comprising a first and second sets of **oligonucleotide** probes as defined by claim 1; wherein each probe in the first probe set from the first group is exactly complementary to a subsequence of a first reference sequence and each probe in the first probe set from the second group is exactly complementary to a subsequence from a second reference sequence.

13. The array of claim 12, wherein the second reference sequence is a mutated form of the first reference sequence.

14. The array of claim 12, wherein each group further comprises third and fourth probe sets, each comprising a corresponding probe for each probe in the first probe set, the probes in the second, third and fourth probe sets being identical to a sequence comprising the corresponding probe from the first probe set or a subsequence of at least three nucleotides thereof that includes the interrogation position, except that the interrogation position is occupied by a different nucleotide in each of the four corresponding probes from the four probe sets.

15. The array of claim 14 that comprises at least forty pairs of first and second probe groups, wherein the probes in the first probe sets from the first groups of the forty pairs are exactly complementary to subsequences from forty respective first reference sequences.

16. A block of **oligonucleotide** probes immobilized on a solid support, comprising: a perfectly matched probe comprising a segment of at least three nucleotides exactly complementary to a subsequence of a reference sequence, the segment having a plurality of interrogation positions respectively corresponding to a plurality of nucleotides in the reference sequence, for each interrogation position, three mismatched probes, each identical to a sequence comprising the perfectly matched probe or a subsequence of at least three nucleotides thereof including the plurality of interrogation positions, except in the interrogation position, which is occupied by a different nucleotide in each of the three mismatched probes and the perfectly matched probe; provided the array lacks a complete set of probes of a given length; wherein the reference sequence is from a CFTR gene.

17. The array of claim 16, wherein the segment of the perfectly matched probe comprises 3-20 interrogation positions corresponding to 3-20 respective nucleotides in the reference sequence.

18. An array of probes immobilized to a solid support comprising at least two blocks of probes, each block as defined by claim 16, a first block comprising a perfectly matched probe comprising a segment exactly complementary to a subsequence of a first reference sequence and a second block comprising a perfectly matched probe comprising a segment exactly complementary to a subsequence of a second reference sequence.

19. The array of claim 18, wherein the first reference sequence is from a wildtype CFTR gene and the second reference sequence is from a mutant CFTR gene.

20. The array of claim 18, comprising at least 10-100 blocks of probes, each comprising a perfectly matched probe comprising a segment exactly complementary to a subsequence of at least 10-100 respective reference sequences.

21. An array of **oligonucleotide** probes immobilized on a solid support, the array comprising at least four probes: a first probe comprising first and second segments, each of at least three nucleotides and exactly complementary to first and second subsequences of a reference sequence, the segments including at least one interrogation position corresponding to a nucleotide in the reference sequence, wherein either (1) the first and second subsequences are noncontiguous, or (2) the first and second subsequences are contiguous and the first and second segments are inverted relative to the complement of the first and second subsequences in the reference sequence; second, third and fourth probes, identical to a sequence comprising the first probe or a subsequence thereof comprising at least three nucleotides from each of the first and second segments, except in the at least one interrogation position, which differs in each of the probes; provided the array lacks a complete set of probes of a given length; wherein the reference sequence is from a CFTR gene.

22. A method of comparing a target nucleic acid with a reference sequence comprising a predetermined sequence of nucleotides, the method comprising: (a) hybridizing a sample comprising the target nucleic acid to an array of **oligonucleotide** probes immobilized on a solid support, the array comprising: (1) a first probe set comprising a plurality of

exactly complementary to a subsequence of the reference sequence, the segment including at least one interrogation position complementary to a corresponding nucleotide in the reference sequence, wherein the reference sequence is from a CFTR gene; (2) a second probe set comprising a corresponding probe for each probe in the first probe set, the corresponding probe in the second probe set being identical to a sequence comprising the corresponding probe from the first probe set or a subsequence of at least three nucleotides thereof that includes the at least one interrogation position, except that the at least one interrogation position is occupied by a different nucleotide in each of the two corresponding probes from the first and second probe sets; wherein, the probes in the first probe set have at least three interrogation positions respectively corresponding to each of at least three nucleotides in the reference sequence, and (b) determining which probes, relative to one another, in the first and second probe sets specifically to the target nucleic acid, the relative specific binding of corresponding probes in the first and second probe sets indicating whether a nucleotide in the target sequence is the same or different from the corresponding nucleotide in the reference sequence.

23. The method of claim 22, wherein the determining step comprises: (1) comparing the relative specific binding of two corresponding probes from the first and second probe sets; (2) assigning a nucleotide in the target sequence as the complement of the interrogation position of the probe having the greater specific binding; (3) repeating (1) and (2) until each nucleotide of interest in the target sequence has been assigned.

24. The method of claim 22, wherein the array further comprises third and fourth probe sets, each comprising a corresponding probe for each probe in the first probe set, the probes in the second, third and fourth probe sets being identical to a sequence comprising the corresponding probe from the first probe set or a subsequence of at least three nucleotides thereof that includes the at least one interrogation position, except that the at least one interrogation position is occupied by a different nucleotide in each of the four corresponding probes from the four probe sets; and the determining step comprises determining which probes, relative to one another, in the first, second, third and fourth probe sets specifically bind to the target nucleic acid, the relative specific binding of corresponding probes in the first, second, third and fourth probe sets indicating whether a nucleotide in the target sequence is the same or different from the corresponding nucleotide in the reference sequence.

25. The method of claim 24, wherein the determining comprises: (1) comparing the relative specific binding of four corresponding probes from the first, second, third and fourth probe sets; (2) assigning a nucleotide in the target sequence as the complement of the interrogation position of the probe having the greatest specific binding; (3) repeating (1) and (2) until each nucleotide of interest in the target sequence has been assigned.

26. A method of comparing a target nucleic acid with a reference sequence comprising a predetermined sequence of nucleotides, the method comprising: (a) hybridizing the target nucleic acid to an array of **oligonucleotide** probes immobilized on a solid support, the array comprising: a perfectly matched probe comprising a segment of at least three nucleotides exactly complementary to a subsequence of a reference sequence, the segment having a plurality of interrogation positions respectively corresponding to a plurality of nucleotides in the reference sequence, wherein the reference sequence is from a CFTR gene; for each interrogation position, three mismatched probes, each identical to a sequence comprising the perfectly matched probe or a subsequence of at least three nucleotides thereof including the plurality of interrogation positions, except in the interrogation position, which is occupied by a different nucleotide in each of the three mismatched probes and the perfectly matched probe; (b) for each interrogation position, (1) comparing the relative specific binding of the three mismatched probes and the perfectly matched probe; (2) assigning a nucleotide in the target sequence as the complement of the interrogation position of the probe having the greatest specific binding.

27. The method of claim 26, wherein the target sequence has an undetermined substitution relative to the reference sequence, and the method assigns a nucleotide to the substitution.

28. A method of comparing a target nucleic acid with a reference sequence comprising a predetermined sequence of nucleotides, the method comprising: hybridizing the target sequence to the array of claim 19; determining which probes in the first group, relative to one another, hybridize to the target sequence, the relative specific binding of the probes indicating whether the target sequence is the same or different

second group, relative to one another, hybridize to the target sequence, the relative specific binding of the probes indicating whether the target sequence is the same or different from the second reference sequence.

29. The method of claim 27, wherein the hybridizing step comprising hybridizing the target sequence and a second target sequence to the array, and the relative specific binding of the probes from the first group indicates that the target is identical to the first reference sequence, and the relative specific binding of the probes from the second group indicates that the second target sequence is identical to the second reference sequence.

30. The method of claim 29, wherein the first and second target sequences are heterozygous alleles.

L5 ANSWER 41 OF 112 USPATFULL on STN

2003:231986 Human cDNAs and proteins and uses thereof.

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US 2003162186 A1 20030828

APPLICATION: US 2002-154678 A1 20020522 (10)

PRIORITY: US 2001-293574P 20010525 (60)

US 2001-298698P 20010615 (60)

US 2001-302277P 20010629 (60)

US 2001-305456P 20010713 (60)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention concerns GENSET polynucleotides and polypeptides. Such GENSET products may be used as reagents in forensic analyses, as chromosome markers, as tissue/cell/organelle-specific markers, in the production of expression vectors. In addition, they may be used in screening and diagnosis assays for abnormal GENSET expression and/or biological activity and for screening compounds that may be used in the treatment of GENSET-related disorders.

CLM What is claimed is:

1. An isolated polynucleotide, comprising a nucleic acid sequence selected from the group consisting of: a) a polynucleotide of an even SEQ ID NO., or of a human cDNA of a deposited clone, encoding at least any single integer from 6 to 500 amino acids of any one odd SEQ ID NO., b) a polynucleotide of an even SEQ ID NO., or of a human cDNA of a deposited clone, encoding the signal peptide sequence of any one odd SEQ ID NO., c) a polynucleotide of an even SEQ ID NO., or of a human cDNA of a deposited clone, encoding a mature polypeptide sequence of any one odd SEQ ID NO., d) a polynucleotide of an even SEQ ID NO., or of a human cDNA of a deposited clone, encoding a full length polypeptide sequence of any one odd SEQ ID NO., e) a polynucleotide of an even SEQ ID NO., or of a human cDNA of a deposited clone, encoding a polypeptide sequence of a biologically active fragment of any one odd SEQ ID NO., f) a polynucleotide encoding a polypeptide sequence of at least any single integer from 6 to 500 amino acids of any one odd SEQ ID NO. or of a polypeptide encoded by a human cDNA of a deposited clone, g) a polynucleotide encoding a polypeptide sequence of a signal peptide of any one odd SEQ ID NO. or of a signal peptide encoded by a human cDNA of a deposited clone, h) a polynucleotide encoding a polypeptide sequence of a mature polypeptide of any one odd SEQ ID NO. or of a mature polypeptide encoded by a human cDNA of a deposited clone, i) a polynucleotide encoding a polypeptide sequence of a full length polypeptide of any one odd SEQ ID NO. or of a mature polypeptide encoded by a human cDNA of a deposited clone, j) a polynucleotide encoding a polypeptide sequence of a biologically polypeptide of any one odd SEQ ID NO., or of a biologically polypeptide encoded by a human cDNA of a deposited clone, k) a polynucleotide of any one of a) through j) further comprising an expression vector, l) a host cell recombinant for a polynucleotide of a) through k) above, m) a non-human transgenic animal comprising the host cell of k), n) a polynucleotide of a) through j) further comprising a physiologically acceptable carrier.

2. A polypeptide comprising an amino acid sequence selected from the group consisting of: a) any single integer from 6 to 500 amino acids of any one odd SEQ ID NO. or of a polypeptide encoded by a human cDNA of a deposited clone; b) a signal peptide sequence of any one odd SEQ ID NO. or encoded by a human cDNA of a deposited clone; c) a mature polypeptide sequence of any one odd SEQ ID NO. or encoded by a human cDNA of a deposited clone; d) a full length polypeptide sequence of any one odd SEQ ID NO. or encoded by a human cDNA of a deposited clone; e) a polypeptide of a) through d) further comprising a physiologically acceptable carrier.

3. A method of making a polypeptide, said method comprising a)

claim 1; b) culturing said population of host cells under conditions conducive to the production of a polypeptide of claim 2 within said host cells; and c) purifying said polypeptide from said population of host cells.

4. A method of making a polypeptide, said method comprising: a) providing a population of cells comprising a polynucleotide encoding the polypeptide of claim 2, operably linked to a promoter; b) culturing said population of cells under conditions conducive to the production of said polypeptide within said cells; and c) purifying said polypeptide from said population of cells.

5. An antibody that specifically binds to the polypeptide of claim 2.

6. A method of binding a polypeptide of claim 2 to an antibody of claim 5, comprising contacting said antibody with said polypeptide under conditions in which antibody can specifically bind to said polypeptide.

7. A method of determining whether a GENSET gene is expressed within a mammal, said method comprising the steps of: a) providing a biological sample from said mammal b) contacting said biological sample with either of: i) a polynucleotide that hybridizes under stringent conditions to the polynucleotide of claim 1; or ii) a polypeptide that specifically binds to the polypeptide of claim 2; and c) detecting the presence or absence of hybridization between said polynucleotide and an RNA species within said sample, or the presence or absence of binding of said polypeptide to a protein within said sample; wherein a detection of said hybridization or of said binding indicates that said GENSET gene is expressed within said mammal.

8. The method of claim 7, wherein said polynucleotide is a **primer**, and wherein said hybridization is detected by detecting the presence of an amplification product comprising the sequence of said **primer**.

9. The method of claim 7, wherein said polypeptide is an antibody.

10. A method of determining whether a mammal has an elevated or reduced level of GENSET gene expression, said method comprising the steps of: a) providing a biological sample from said mammal; and b) comparing the amount of the polypeptide of claim 2, or of an RNA species encoding said polypeptide, within said biological sample with a level detected in or expected from a control sample; wherein an increased amount of said polypeptide or said RNA species within said biological sample compared to said level detected in or expected from said control sample indicates that said mammal has an elevated level of said GENSET gene expression, and wherein a decreased amount of said polypeptide or said RNA species within said biological sample compared to said level detected in or expected from said control sample indicates that said mammal has a reduced level of said GENSET gene expression.

11. A method of identifying a candidate modulator of a GENSET polypeptide, said method comprising: a) contacting the polypeptide of claim 2 with a test compound; and b) determining whether said compound specifically binds to said polypeptide; wherein a detection that said compound specifically binds to said polypeptide indicates that said compound is a candidate modulator of said GENSET polypeptide.

12. The method of claim 11, further comprising testing the biological activity of said GENSET polypeptide in the presence of said candidate modulator, wherein an alteration in the biological activity of said GENSET polypeptide in the presence of said compound in comparison to the activity in the absence of said compound indicates that the compound is a modulator of said GENSET polypeptide.

13. A method for the production of a pharmaceutical composition comprising a) identifying a modulator of a GENSET polypeptide using the method of claim 11; and b) combining said modulator with a physiologically acceptable carrier.

L5 ANSWER 42 OF 112 USPATFULL ON STN

2003:226321 Vaccine for the prophylactic or therapeutic immunization against hiv.

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US 2003158134 A1 20030821

APPLICATION: US 2002-203013 A1 20020731 (10)

WO 2001-EP944 20010129

PRIORITY: GB 2000-2200 20000131

GB 2000-9336 20000414

GB 2000-13806 20000606

WO 2000-EP5998 20000628

DOCUMENT TYPE: Utility; APPLICATION.

AB The invention provides the use of a) an HIV Tat protein or polynucleotide; or b) an HIV Nef protein or polynucleotide; or c) an HIV Tat protein or polynucleotide linked to an HIV Nef protein or polynucleotide (Nef-Tat); and an HIV gpl20 protein or polynucleotide in the manufacture of a vaccine for the prophylactic or therapeutic immunisation of humans against HIV.

CLM What is claimed is:

1. Use of a) an HIV Tat protein or polynucleotide; or b) an HIV Nef protein or polynucleotide; or c) an HIV Tat protein or polynucleotide linked to an HIV Nef protein or polynucleotide (Nef-Tat); and an HIV gpl20 protein or polynucleotide in the manufacture of a vaccine for the prophylactic or therapeutic immunisation of humans against HIV, wherein the Tat, Nef or Nef-Tat act in synergy with gpl20 in the treatment or prevention of HIV.
2. Use as claimed in claim 1 wherein the vaccine in use reduces the HIV viral load in HIV infected humans.
3. Use as claimed in claims 1 or 2 wherein the vaccine in use results in a maintenance of CD4+ levels over those levels found in the absence of vaccination with HIV Tat, Nef or Nef-Tat and HIV gpl20.
4. Use as claimed in any one of claims 1-3 wherein the vaccine further comprises an antigen selected from the group consisting of: gag, rev, vif, vpr, vpu.
5. Use as claimed in any one of claims 1-4 wherein the Tat protein is a mutated protein.
6. Use as claimed in any one of claims 1-5 wherein the Tat, Nef or Nef-Tat protein is reduced.
7. Use as claimed in any one of claims 1-6 wherein the Tat, Nef or Nef-Tat protein is carbamidomethylated.
8. Use as claimed in any one of claims 1-5 wherein the Tat, Nef or Nef-Tat protein is oxidised.
9. Use as claimed in any one of claims 1-8 which additionally comprises an adjuvant.
10. Use as claimed in claim 9 wherein the adjuvant is a TH1 inducing adjuvant.
11. Use as claimed in claim 9 or claim 10 wherein the adjuvant comprises monophosphoryl lipid A or a derivative thereof such as 3-de-O-acylated monophosphoryl lipid A.
12. Use as claimed in any one of claims 9-11 additionally comprising a saponin adjuvant.
13. Use as claimed in any one of claims 9-12 additionally comprising an oil in water emulsion.
14. Use as claimed in claim 9 or claim 10 wherein the adjuvant comprises CpG motif-containing **oligonucleotides**.
15. Use as claimed in claim 14 further comprising an aluminium salt.
16. Use of a) an HIV Tat protein or polynucleotide; or b) an HIV Nef protein or polynucleotide; or c) an HIV Tat protein or polynucleotide linked to an HIV Nef protein or polynucleotide; and an HIV gpl20 protein or polynucleotide in the manufacture of a vaccine suitable for a prime-boost delivery for the prophylactic or therapeutic immunisation of humans against HIV.
17. A method of immunising a human against HIV by administering to the human a vaccine comprising HIV Tat or HIV Nef or HIV Nef-Tat in combination with HIV gpl20 proteins or polynucleotides encoding them.
18. A vaccine composition for human use which vaccine composition comprises HIV Tat or HIV Nef or HIV Nef-Tat in combination with HIV gpl20 proteins or polynucleotides encoding them.
- 19 A schedule for vaccination with gpl20, nef and tat comprising the sequential administration of protein antigens and DNA encoding gpl20, nef and tat.
- 20 A schedule according to claim 19, wherein the protein antigens are injected once or several times followed by one or more DNA administrations.

one or more administrations followed by one or more protein administrations.

22 Use of (a) a composition comprising gp120 Nef, Tat and gp120 proteins; and (b) a composition comprising gp120, Nef and Tat DNA in the preparation of a medicament for treatment of HIV, wherein (a) and (b) may be used separately, in any order or together.

23 Use of gp120, nef and tat protein antigens in the preparation of a medicament for the treatment of HIV in an individual to whom DNA encoding gp120, nef and tat protein antigens has been administered.

24 Use of DNA encoding gp120, nef and tat protein antigens in the preparation of a medicament for the treatment of HIV in an individual to whom gp120, nef and tat protein antigens have been administered.

L5 ANSWER 43 OF 112 USPTAFULL on STN

2003:225673 Human cDNAs and proteins and uses thereof.

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US 2003157485 A1 20030821

APPLICATION: US 2001-992095 A1 20011113 (9)

PRIORITY: WO 2001-IB1715 20010806

US 2001-305456P 20010713 (60)

US 2001-302277P 20010629 (60)

US 2001-298698P 20010615 (60)

US 2001-293574P 20010525 (60)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention concerns GENSET polynucleotides and polypeptides. Such GENSET products may be used as reagents in forensic analyses, as chromosome markers, as tissue/cell/organelle-specific markers, in the production of expression vectors. In addition, they may be used in screening and diagnosis assays for abnormal GENSET expression and/or biological activity and for screening compounds that may be used in the treatment of GENSET-related disorders.

CLM What is claimed is:

1. An isolated polynucleotide, comprising a nucleic acid sequence selected from the group consisting of: a) a polynucleotide of SEQ ID NO: 53, or of a human cDNA of deposited clone 182-14-3-0-C12-F, encoding at least any single integer from 6 to 500 amino acids of SEQ ID NO: 54; b) a polynucleotide of SEQ ID NO: 53, or of a human cDNA of deposited clone 182-14-3-0-C12-F, encoding the signal peptide sequence of SEQ ID NO: 54; c) a polynucleotide of SEQ ID NO: 53, or of a human cDNA of deposited clone 182-14-3-0-C12-F, encoding a mature polypeptide sequence of SEQ ID NO: 54; d) a polynucleotide of SEQ ID NO: 53, or of a human cDNA of deposited clone 182-14-3-0-C12-F, encoding a full length polypeptide sequence of SEQ ID NO: 54; e) a polynucleotide of SEQ ID NO: 53, or of a human cDNA of deposited clone 182-14-3-0-C12-F, encoding a polypeptide sequence of a biologically active fragment of SEQ ID NO: 54; f) a polynucleotide encoding a polypeptide sequence of at least any single integer from 6 to 500 amino acids of SEQ ID NO: 54 or of a polypeptide encoded by a human cDNA of deposited clone 182-14-3-0-C12-F; g) a polynucleotide encoding a polypeptide sequence of a signal peptide of SEQ ID NO: 54 or of a signal peptide encoded by a human cDNA of deposited clone 182-14-3-0-C12-F; h) a polynucleotide encoding a polypeptide sequence of a mature polypeptide of SEQ ID NO: 54 or of a mature polypeptide encoded by a human cDNA of deposited clone 182-14-3-0-C12-F; i) a polynucleotide encoding a polypeptide sequence of a full length polypeptide of SEQ ID NO: 54 or of a mature polypeptide encoded by a human cDNA of deposited clone 182-14-3-0-C12-F; j) a polynucleotide encoding a polypeptide sequence of a biologically active polypeptide of SEQ ID NO: 54, or of a biologically active polypeptide encoded by a human cDNA of deposited clone 182-14-3-0-C12-F; k) a polynucleotide of any one of a) through j) further comprising an expression vector; l) a host cell recombinant for a polynucleotide of a) through k) above; m) a non-human transgenic animal comprising the host cell of k); and n) a polynucleotide of a) through j) further comprising a physiologically acceptable carrier.

2. A polypeptide comprising an amino acid sequence selected from the group consisting of: a) any single integer from 6 to 500 amino acids of SEQ ID NO: 54 or of a polypeptide encoded by a human cDNA of deposited clone 182-14-3-0-C12-F; b) a signal peptide sequence of SEQ ID NO: 54 or encoded by a human cDNA of deposited clone 182-14-3-0-C12-F; c) a mature polypeptide sequence of SEQ ID NO: 54 or encoded by a human cDNA of deposited clone 182-14-3-0-C12-F; d) a full length polypeptide sequence of SEQ ID NO: 54 or encoded by a human cDNA of deposited clone 182-14-3-0-C12-F; and e) a polypeptide of a) through d) further comprising a physiologically acceptable carrier.

3. A method of making a polypeptide, said method comprising: a) providing a population of host cells comprising the polynucleotide of claim 1; b) culturing said population of host cells under conditions conducive to the production of a polypeptide of claim 2 within said host cells; and c) purifying said polypeptide from said population of host cells.
4. A method of making a polypeptide, said method comprising: a) providing a population of cells comprising a polynucleotide encoding the polypeptide of claim 2, operably linked to a promoter; b) culturing said population of cells under conditions conducive to the production of said polypeptide within said cells; and c) purifying said polypeptide from said population of cells.
5. An antibody that specifically binds to the polypeptide of claim 2.
6. A method of binding a polypeptide of claim 2 to an antibody of claim 5, comprising contacting said antibody with said polypeptide under conditions in which antibody can specifically bind to said polypeptide.
7. A method of determining whether a Plasmin gene is expressed within a mammal, said method comprising the steps of: a) providing a biological sample from said mammal; b) contacting said biological sample with either of: i) a polynucleotide that hybridizes under stringent conditions to the polynucleotide of claim 1; or ii) a polypeptide that specifically binds to the polypeptide of claim 2; and c) detecting the presence or absence of hybridization between said polynucleotide and an RNA species within said sample, or the presence or absence of binding of said polypeptide to a protein within said sample; wherein a detection of said hybridization or of said binding indicates that said Plasmin gene is expressed within said mammal.
8. The method of claim 7, wherein said polynucleotide is a **primer**, and wherein said hybridization is detected by detecting the presence of an amplification product comprising the sequence of said **primer**.
9. The method of claim 7, wherein said polypeptide is an antibody.
10. A method of determining whether a mammal has an elevated or reduced level of a Plasmin gene expression, said method comprising the steps of: a) providing a biological sample from said mammal; and b) comparing the amount of the polypeptide of claim 2, or of an RNA species encoding said polypeptide, within said biological sample with a level detected in or expected from a control sample; wherein an increased amount of said polypeptide or said RNA species within said biological sample compared to said level detected in or expected from said control sample indicates that said mammal has an elevated level of said Plasmin gene expression, and wherein a decreased amount of said polypeptide or said RNA species within said biological sample compared to said level detected in or expected from said control sample indicates that said mammal has a reduced level of said Plasmin gene expression.
11. A method of identifying a candidate modulator of a Plasmin polypeptide, said method comprising: a) contacting the polypeptide of claim 2 with a test compound; and b) determining whether said compound specifically binds to said polypeptide; wherein a detection that said compound specifically binds to said polypeptide indicates that said compound is a candidate modulator of said Plasmin polypeptide.
12. The method of claim 11, further comprising testing the biological activity of said Plasmin polypeptide in the presence of said candidate modulator, wherein an alteration in the biological activity of said Plasmin polypeptide in the presence of said compound in comparison to the activity in the absence of said compound indicates that the compound is a modulator of said Plasmin polypeptide.
13. A method for the production of a pharmaceutical composition comprising a) identifying a modulator of a Plasmin polypeptide using the method of claim 11; and b) combining said modulator with a physiologically acceptable carrier.

L5 ANSWER 44 OF 112 USPATFULL on STN

2003:220440 Endothelial cell-leukocyte adhesion molecules (ELAMs) and molecules involved in leukocyte adhesion (MILAs).

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US 2003153731 A1 20030814

AB DNA sequences encoding endothelial cell-leukocyte adhesion molecules ELAMs, methods for producing such molecules, and ELAMs (including the specific molecules ELAM1 and VCAM1 and 1b) essentially free of normally associated animal proteins are disclosed. Antibodies against ELAMs are also disclosed.

DNA sequences encoding molecules involved in leukocyte adhesion (MILAs), methods for producing such molecules and MILAs (including the specific molecule, CDX) essentially free of normally associated animal proteins are also disclosed. Antibody preparations which are reactive for MILAs and also disclosed.

We disclose DNA sequences designated clone 7.2 and clone 1, which cause cells transformed with them to express 1,3-fucosyl transferases and which are involved in CDX expression. We also disclose protein 7.2 and protein 1 which are encoded by clone 7.2 and clone 1, respectively.

We also disclose Pseudo-X and Pseudo-X₂, proteins which cause COS cells and CHO cells to bind to ELAM1 and to be recognized by α -CDX antibodies.

Methods for identifying molecules which inhibit binding of leukocytes to endothelial cells, methods for inhibiting leukocyte binding to endothelial cells, and methods for detecting acute inflammation are disclosed.

CLM What is claimed is:

1. A DNA sequence encoding an endothelial cell-leukocyte adhesion molecule (ELAM) or a fragment thereof selected from the group consisting of: (a) the ELAM1 DNA sequence of FIG. 1 from nucleotide number 141 to number 1970; (b) the ELAM1 DNA sequence of FIG. 1 from nucleotide number 144 to number 1970; (c) a DNA sequence encoding an amino acid sequence of a mature ELAM1; (d) the DNA sequence of FIG. 1 from nucleotide number 204 to number 1970, optionally including an ATG start codon at its 5' end; (e) a DNA sequence encoding a soluble ELAM1; (f) DNA sequences that hybridize to any of the foregoing DNA sequences under standard hybridization conditions; and (g) DNA sequences that code on expression for an amino acid sequence encoded by any of the foregoing DNA sequences.

2. A recombinant DNA molecule comprising a DNA sequence encoding an endothelial cell-leukocyte adhesion molecule (ELAM) or a fragment thereof, wherein said DNA sequence is selected from the group consisting of: (a) the ELAM1 DNA sequence of FIG. 1 from nucleotide number 141 to number 1970; (b) the ELAM1 DNA sequence of FIG. 1 from nucleotide number 144 to number 1970; (c) a DNA sequence encoding an amino acid sequence of a mature ELAM1; (d) the DNA sequence of FIG. 1 from nucleotide number 204 to number 1970, optionally including an ATG start codon at its 5' end; (e) a DNA sequence encoding a soluble ELAM1; (f) DNA sequences that hybridize to any of the foregoing DNA sequences under standard hybridization conditions; and (g) DNA sequences that code on expression for an amino acid sequence encoded by any of the foregoing DNA sequences.

3. A recombinant DNA molecule according to claim 2 wherein said DNA sequence is operatively linked to an expression control sequence.

4. The recombinant DNA molecule of claim 3, wherein said expression control sequence is selected from the group consisting of the early or late promoters of SV40 or adenovirus, the lac system, the try system, the TAC system, the TRC system, the major operator and promoter regions of phage λ , the control regions of fd coat protein, the promoter for 3-phosphoglycerate kinase, the promoters of acid phosphatase and the promoters of the yeast α -mating factors.

5. A recombinant DNA molecule according to claim 3 comprising plasmid ELAM pCDM8 clone 6.

6. A unicellular host transformed with a recombinant DNA molecule comprising a DNA sequence encoding an endothelial cell-leukocyte adhesion molecule (ELAM) or fragment thereof, wherein said DNA sequence is selected from the group consisting of: (a) the ELAM1 DNA sequence of FIG. 1 from nucleotide number 141 to number 1970; (b) the ELAM1 DNA sequence of FIG. 1 from nucleotide number 144 to number 1970; (c) a DNA sequence encoding an amino acid sequence of a mature ELAM1; (d) the DNA sequence of FIG. 1 from nucleotide number 204 to number 1970 optionally containing an ATG start codon at its 5' end; (e) a DNA sequence encoding a soluble ELAM1; (f) DNA sequences that hybridize to any of the foregoing DNA sequences under standard hybridization conditions; and (g) DNA sequences that code on expression for an amino acid sequence encoded by any of the foregoing DNA sequences; wherein said DNA sequence

7. A unicellular host according to claim 6, wherein said expression control sequence is selected from the group consisting of the early or late promoters of SV40 or adenovirus, the lac system, the trp system, the TAC system, the TRC system, the major operator and promoter regions of phage A, the control regions of fd coat protein, the promoter for 3-phosphoglycerate kinase, the promoters of acid phosphatase and the promoters of the yeast a-mating factors.

8. A transformed host according to claim 6, comprising plasmid ELAM pCDM8 clone 6.

9. A transformed host according to claim 6 wherein the unicellular host is selected from the group consisting of E.coli, Pseudomonas, Bacillus, Streptomyces, yeasts, CHO, R1.1, B-W, L-M, COS 1, COS 7, BSC1, BSC40, and BMT10 cells, plant cells, insect cells, and human cells in tissue culture.

10. A method for producing ELAM1 comprising the step of culturing a transformed host according to claim 7.

11. A cytokine-inducible expression control sequence derived from the nucleotide sequence of FIG. 7.

12. The cytokine-inducible expression control sequence of claim 11 comprising nucleotides 740-1307 of FIG. 7.

13. An ELAM or fragment thereof essentially free of normally associated animal proteins selected from the group consisting of ELAM1, the lectin-like domain of ELAM1, the EGF-like domain of ELAM1, the consensus cysteine repeat unit of ELAM1, soluble ELAM1, mature ELAM1, and ELAM1 fragments capable of binding to an ELAM1 ligand.

14. A molecule according to claim 13 comprising the amino acid sequence of FIG. 1 from amino acid number 22 (Trp) to amino acid number 609 (Leu) optionally including an N-terminal methionine residue.

15. Hybridoma CDB.BB11.BC6 anti-ELAM1.

16. The monoclonal antibodies produced by hybridoma CDB.BB11.BC6 anti-ELAM1.

17. A DNA sequence encoding a MILA for ELAM1 or fragment thereof.

18. The DNA sequence according to claim 17 wherein the MILA is CDX.

19. The DNA sequence according to claim 17 wherein the MILA is an ELAM1 ligand.

20. A recombinant DNA molecule comprising a DNA sequence encoding a MILA for ELAM1 or a fragment thereof.

21. The recombinant DNA molecule of claim 20, wherein the MILA is CDX.

22. The recombinant DNA molecule of claim 20, wherein the MILA is an ELAM1 ligand.

23. The recombinant DNA molecule of claim 20 wherein said DNA sequence is operatively linked to an expression control sequence.

24. A unicellular host transformed with a recombinant DNA molecule comprising a DNA sequence encoding a MILA for ELAM1 or fragment thereof.

25. The unicellular host of claim 24, wherein the MILA is CDX.

26. The unicellular host of claim 24, wherein the MILA is an ELAM1 ligand.

27. A unicellular host of claim 24 selected from the group consisting of E.coli, Pseudomonas, Bacillus, Streptomyces, yeasts, CHO, R1.1, B-W, L-M, COS 1, COS 7, BSC1, BSC40, BMT10, insect cells, plant cells, and human cells in tissue culture.

28. A MILA for ELAM1 or a fragment thereof, substantially free of normally associated animal proteins.

29. The MILA of claim 28 comprising CDX.

30. The MILA according to claim 28 comprising an ELAM1 ligand.

31. A fragment of a MILA according to claim 28 that binds to ELAM1.

carbohydrate.

33. Hybridoma SGB_{3B4}.

34. The monoclonal antibodies produced by hybridoma SGB_{3B4}.

35. An antibody preparation that is reactive to a MILA for ELAM1 but non-reactive to other proteins on the leukocyte cell surface.

36. The antibody preparation of claim 35 wherein the MILA is CDX.

37. The antibody preparation of claim 35 consisting essentially of monoclonal antibodies.

38. A method for producing an antibody preparation reactive to a MILA for ELAM1 comprising the step of immunizing an organism with CDX or an antigenic fragment thereof.

39. A DNA sequence encoding an endothelial cell-leukocyte adhesion molecule or fragment thereof selected from the group consisting of (a) the VCAM1 DNA sequence of FIG. 3 from nucleotide number 107 to number 2047; (b) the VCAM1 DNA sequence of FIG. 3 from nucleotide number 110 to number 2047; (c) a DNA sequence encoding an amino acid sequence of a mature VCAM1; (d) the VCAM1 DNA sequence of FIG. 3 from nucleotide number 179 to 2047, optionally including an ATG start-codon at its 5' end; (e) a DNA sequence encoding a soluble VCAM1; (f) the VCAM1b DNA sequence of FIG. 4 from nucleotide number 100 to number 2316; (g) the VCAM1b DNA sequence of FIG. 4 from nucleotide number 103 to number 2316; (h) a DNA sequence encoding an amino acid sequence of mature VCAM1b; (i) a DNA sequence encoding a soluble VCAM1b; (j) the VCAM1b DNA sequence of FIG. 4 from nucleotide number 172 to 2316, optionally including an ATG start codon at its 5' end; (k) DNA sequences that hybridize to any of the foregoing DNA sequences under standard hybridization conditions; and (l) DNA sequences that code on expression for an amino acid sequence encoded by any of the foregoing DNA sequences.

40. A recombinant DNA molecule comprising a DNA sequence encoding an endothelial cell-leukocyte adhesion molecule (ELAM) or a fragment thereof wherein said DNA sequence is selected from the group consisting of: (a) the VCAM1 DNA sequence of FIG. 3 from nucleotide number 107 to number 2047; (b) the VCAM1 DNA sequence of FIG. 3 from nucleotide number 110 to number 2047; (c) a DNA sequence encoding an amino acid sequence of mature VCAM1; (d) the VCAM1b DNA sequence of FIG. 4 from nucleotide number 172 to 2316, optionally including an ATG start codon at its 5' end; (e) a DNA sequence encoding a soluble VCAM1; (f) the VCAM1b DNA sequence of FIG. 4 from nucleotide number 100 to number 2316; (g) the VCAM1b DNA sequence of FIG. 4 from nucleotide number 103 to number 2316; (h) a DNA sequence encoding an amino acid sequence of mature VCAM1b; (i) a DNA sequence encoding a soluble VCAM1b; (j) the VCAM1b DNA sequence of FIG. 4 from nucleotide number 172 to 2316, optionally including an ATG start codon at its 5' end; (k) DNA sequences that hybridize to any of the foregoing DNA sequences under standard hybridization conditions; and (l) DNA sequences that code on expression for an amino acid sequence encoded by any of the foregoing DNA sequences.

41. The recombinant DNA molecule of claim 40 wherein said DNA sequence is operatively linked to an expression control sequence.

42. The recombinant DNA molecule of claim 41, wherein said expression control sequence is selected from the group consisting of the early or late promoters of SV40 or adenovirus, the lac system, the trp system, the TAC system, the TRC system, the major operator and promoter regions of phage λ , the control regions of fd coat protein, the promoter of 3-phosphoglycerate kinase, the promoters of acid phosphatase and the promoters of yeast a-mating factors.

43. A recombinant DNA molecule according to claim 41, comprising plasmid AM pCDM 8 clone 41 or plasmid VCAM 1B clone 1E11 pCDM8.

44. A unicellular host transformed with a recombinant DNA molecule comprising a DNA sequence encoding an endothelial cell-leukocyte adhesion molecule (ELAM) or a fragment thereof, wherein said DNA sequence is selected from the group consisting of: (a) the VCAM1 DNA sequence of FIG. 3 from nucleotide number 107 to number 2047; (b) the VCAM1 DNA sequence of FIG. 3 from nucleotide number 110 to number 2047; (c) a DNA sequence encoding an amino acid sequence of mature VCAM1; (d) the VCAM1b DNA sequence of FIG. 4 from nucleotide number 172 to 2316, optionally including an ATG start codon at its 5' end; (e) a DNA sequence encoding a soluble VCAM1; (f) the VCAM1b DNA sequence of FIG. 4 from nucleotide number 100 to number 2316; (g) the VCAM1b DNA

sequence encoding an amino acid sequence of mature VCAM1b; (i) a DNA sequence encoding a soluble VCAM1b; (j) the VCAM1b DNA sequence of FIG. 4 from nucleotide number 172 to 2316, optionally including an ATG start codon at its 5' end; (k) DNA sequences that hybridize to any of the foregoing DNA sequences under standard hybridization conditions; and (l) DNA sequences that code on expression for an amino acid sequence encoded by any of the foregoing DNA sequences; and wherein said DNA sequence is operatively linked to an expression control sequence.

45. A unicellular host according to claim 44, wherein the recombinant DNA molecule comprises plasmid AM pCDM 8 clone 41 or clone VCAM 1B pCDM8 clone 1E11.

46. A unicellular host of claim 44, selected from the group consisting of E.coli, Pseudomonas, Bacillus, Streptomyces, yeasts, CHO, R1.1, B-W, L-M, COS 1, COS 7, BSC1, BSC40, and BMT10, insect cells, plant cells, and human cells in tissue culture.

47. A method for producing VCAM1 comprising the step of culturing a unicellular host according to claim 44.

48. VCAM1 or VCAM1b or a fragment thereof essentially free of normally associated animal proteins.

49. A VCAM1 or VCAM1b polypeptide selected from the group consisting of domain 1 of VCAM1, domain 2 of VCAM1, domain 3 of VCAM1, domain 4 of VCAM1, domain 5 of VCAM1, domain 6 of VCAM1, domain 3 of VCAM1b, domain 3B of VCAM1b, domain 4 of VCAM1b, and combinations thereof.

50. A VCAM1 according to claim 48, comprising the amino acid sequence of FIG. 3 from amino acid number 25 to amino acid number 647, optionally including an N-terminal methionine residue.

51. An antibody preparation that is reactive for VCAM1 or VCAM1b but non-reactive for other adhesion molecules expressed on the endothelial cell surface.

52. The antibody preparation of claim 51 wherein said antibody preparation consists essentially of monoclonal antibodies.

53. A hybridoma producing monoclonal antibodies that recognize VCAM1.

54. A method for producing antibodies which recognize VCAM1 or VCAM1b, comprising the step of immunizing an organism with VCAM1 or VCAM1b or an antigenic fragment thereof.

55. A method for identifying molecules which inhibit binding of leukocytes to endothelial cells comprising the steps of: (a) contacting a molecule with an ELAM or with ELAM-expressing cells to create a first mixture; (b) contacting said first mixture with an ELAM ligand or with cells expressing a MILA or another molecule that binds to an ELAM, to create a second mixture; and (c) testing said second mixture for the amount of said ELAM or ELAM expressing cells bound to said ELAM ligand or cells expressing a MILA or another molecule that binds to an ELAM.

56. A method for identifying molecules which inhibit binding of leukocytes to endothelial cells comprising the steps of: (a) contacting a molecule with an ELAM ligand or with cells expressing a MILA or a molecule that binds to an ELAM, to create a first mixture; (b) contacting said first mixture with an ELAM or with ELAM expressing cells to create a second mixture; and (c) testing said second mixture for the amount of said ELAM ligand or cells expressing a MILA or a molecule that binds to an ELAM, bound to said ELAM or ELAM-expressing cells.

57. The method of claim 55 or 56, wherein the ELAM is ELAM1.

58. The method of claim 55 or 56, wherein the ELAM ligand is ELAM1 ligand.

59. The method of claim 55 or 56, wherein the cells express a molecule selected from the group consisting of CDX, Pseudo-X and Pseudo-X₂.

60. The method of claim 55 or 56, wherein the ELAM is VCAM1 or VCAM1b.

61. The method of claim 55 or 56, wherein the MILA is a VCAM1 or VCAM1b ligand.

62. The method of claim 55 or 56 wherein the MILA is VLA4.

63. A method of inhibiting adhesion between leukocytes and endothelial cells in a system containing them comprising the step of introducing an effective amount of an inhibitory agent into said system, wherein said

fragments thereof capable of binding to ELAM ligands, antibodies recognizing MILAs, ELAM ligands or fragments thereof capable binding to ELAMs, carbohydrates binding to ELAMs, and antibodies recognizing ELAMs.

64. The method of claim 63, wherein the inhibitory agent is ELAM1 or a fragment of an ELAM selected from the group consisting of the lectin-like domain of an ELAM1, the EGF-like domain of ELAM1, the consensus cysteine repeat of ELAM1, and a soluble ELAM1.

65. The method of claim 63, wherein the inhibitory agent is a preparation of monoclonal antibodies recognizing ELAM1 ligand.

66. The method of claim 63, wherein the inhibitory agent is a monoclonal antibody which recognizes CDX.

67. The method of claim 66, wherein the monoclonal antibody is that produced by hybridoma SGB_{3B4}.

68. The method of claim 63, wherein said inhibitory agent is an ELAM1 ligand or a fragment thereof capable of binding to ELAM1.

69. The method of claim 63, wherein the inhibitory agent is a monoclonal antibody recognizing ELAM1.

70. The method of claim 69 wherein the monoclonal antibody is that produced by hybridoma CDB.BB11.BC6 anti-ELAM1.

71. The method of claim 63 wherein said inhibitory agent is selected from the group consisting of VCAM1, VCAM1b and fragments thereof that bind to VLA4.

72. The method of claim 63 wherein said inhibitory agent is a monoclonal antibody which recognizes a VCAM1 ligand.

73. The method of claim 63 wherein said inhibitory agent is a VCAM1 ligand or a fragment thereof that binds to VCAM1 or VCAM1b.

74. The method of claim 73 wherein the VCAM1 ligand is VLA4.

75. The method of claim 63, wherein said inhibitory agent is a monoclonal antibody that recognizes VCAM1 or VCAM1b.

76. A method of detecting inflammation comprising the step of administering a detectably labelled compound selected from the group consisting of ELAM ligands, ELAM-binding fragments of an ELAM ligand, and antibodies which recognize an ELAM.

77. A method of detecting inflammation comprising the steps of: (a) contacting a sample of blood, serum, or other bodily fluid with detectably labelled ELAM ligands, ELAM-binding fragments of an ELAM ligand, or antibodies which recognize an ELAM, to create a mixture, and (b) testing said mixture for the amount of ELAM ligand, ELAM-binding fragment of an ELAM ligand, or antibodies bound to an ELAM.

78. The method of claims 76 or 77, wherein the ELAM ligand is ELAM1 ligand.

79. The method of claims 76 or 77 wherein the ELAM ligand is a VCAM1 ligand or VCAM1b ligand.

80. The method of claim 79 wherein the VCAM1 ligand is VLA4.

81. The method of claims 76 or 77 wherein the antibodies are those produced by hybridoma CDB.B11.BC6 anti-ELAM1.

82. A recombinant DNA molecule coding on expression for an ELAM/immunoglobulin fusion protein comprising a DNA sequence coding on expression for an ELAM or fragment thereof and a DNA sequence coding on expression for the constant region of an immunoglobulin molecule.

83. The DNA sequence of claim 82 wherein the ELAM is ELAM1, VCAM1 or VCAM1b.

84. A recombinant DNA molecule according to claim 82 comprising VCAM1 domains 1-3 and the constant regions of human immunoglobulin C-gamma-1.

85. An antisense nucleic acid against an ELAM or MILA mRNA comprising a nucleic acid sequence hybridizing to said mRNA.

86. The antisense **oligonucleotide** of claim 85 which binds to the initiation codon of any of said mRNAs.

VCAM1 or VCAM1b.

88. The antisense nucleic acid of claim 85, wherein the MILA is an ELAM1 ligand.

89. The antisense nucleic acid of claim 85, wherein the MILA is CDX.

90. The antisense nucleic acid of claim 85, wherein the MILA is VLA4.

91. The antisense nucleic acid of claim 85 comprising DNA.

92. An antisense nucleic acid of claim 85, comprising RNA.

93. The antisense nucleic acid of claim 85 having the DNA sequence 5' CCC AGG CAT TTT AAG.

94. A recombinant DNA molecule having a DNA sequence which, on transcription, produces an antisense ribonucleic acid against an ELAM or MILA mRNA, said antisense ribonucleic acid comprising a nucleic acid sequence hybridizing to said mRNA.

95. An ELAM-producing or MILA-producing cell line transfected with a recombinant DNA molecule having a DNA sequence which, on transcription, produces an antisense ribonucleic acid against an ELAM or MILA mRNA, said antisense ribonucleic acid comprising a nucleic acid sequence hybridizing to said mRNA.

96. A method for creating a cell line which exhibits reduced expression of an ELAM or MILA comprising transfecting an ELAM-producing or MILA-producing cell line with a recombinant DNA molecule having a DNA sequence which, upon transcription, produces an antisense ribonucleic acid against an ELAM or MILA mRNA, said antisense ribonucleic acid comprising a nucleic acid sequence hybridizing said mRNA.

97. A method of treating inflammation comprising the step of administering an amount of an antisense nucleic acid according to claim 82 effective to reduce production of one or more ELAMs or MILAs.

98. A ribozyme which cleaves an ELAM or MILA mRNA.

99. A ribozyme according to claim 98 that cleaves ELAM1, VCAM1 or VCAM1b mRNA.

100. A ribozyme according to claim 98 that cleaves an ELAM ligand mRNA.

101. A ribozyme according to claim 98 that cleaves CDX mRNA.

102. A ribozyme according to claim 98 that cleaves VLA4 mRNA.

103. A ribozyme according to claim 98 further comprising a Tetrahymena-type ribozyme.

104. A ribozyme according to claim 98 further comprising a hammerhead-type ribozyme.

105. A recombinant DNA molecule comprising a DNA sequence which, upon transcription, produces a ribozyme that cleaves an ELAM or MILA mRNA.

106. The ribozyme according to claim 98 comprising the RNA sequence 5' AAGGAUCACC UGAUGAGUCC GUGAGGACGA AACCAUCUU.

107. An ELAM-producing or MILA-producing cell line transfected with a recombinant DNA molecule comprising a DNA sequence which, upon transcription, produces a ribozyme that cleaves an ELAM or MILA mRNA.

108. A method for creating a cell line which exhibits reduced expression of an ELAM or MILA comprising transfecting an ELAM-producing or MILA-producing cell line with a recombinant DNA molecule that produces on transcription a ribozyme that cleaves an ELAM or MILA mRNA.

109. A method of treating inflammation comprising the step of administering an amount of a ribozyme that cleaves an ELAM mRNA or an ELAM ligand mRNA effective to reduce the production of ELAM or ELAM ligand.

110. An anti-idiotypic antibody preparation reactive to an ELAM or MILA.

111. An anti-idiotypic antibody preparation of claim 110 reactive to ELAM1, VCAM1 or VCAM1b.

112. An anti-idiotypic antibody preparation of claim 110 reactive to an ELAM1 ligand.

113. An anti-idiotypic antibody preparation of claim 110 reactive to VLA4.
114. An anti-idiotypic antibody preparation of claim 110 reactive to CDX.
115. An anti-idiotypic antibody preparation of claim 110 reactive to domains 1, 2, 3, 4, 5 or 6 of VCAM1 or domains 3, 3B or 4 of VCAM1b.
116. A method for identifying an ELAM or ELAM-ligand which binds to any one of ELAM1, CDX, VCAM1, VCAM1b, or VLA4, comprising the steps of: (a) screening a mixture of proteins for proteins that bind to anti-idiotypic antibodies recognizing antibodies that recognize any one of ELAM1, CDX, VCAM1, VCAM1b, or VLA4; (b) isolating those molecules which bind to said anti-idiotypic antibodies; and (c) testing the ability of those proteins to bind to ELAM1, CDX, VCAM1, VCAM1b, or VLA4.
117. A method for identifying ELAM ligands which bind to any of the domains of VCAM1 or VCAM1b comprising the steps of: (a) screening a mixture of proteins for proteins that bind to anti-idiotypic antibodies recognizing antibodies which recognize any of said domains of VCAM1 or VCAM1b; (b) isolating those proteins that bind to said anti-idiotypic antibodies; and (c) testing the ability of those proteins to bind to any of said domains of VCAM1 or VCAM1b.
118. A method for inhibiting VCAM1 or VCAM1b expression comprising the step of administering an effective dose of an antibody which recognizes IL-1, TNF, or IFN- γ .
119. A radioimmunoconjugate comprising an antibody conjugated to a nuclide, said antibody selected from the group consisting of antibodies recognizing VCAM1, antibodies recognizing VCAM1b, monoclonal antibody SGB3B4 and monoclonal antibody BB11.
120. The radioimmunoconjugate of claim 119 wherein the nuclide is selected from the group consisting of ^{125}I , ^{90}Y , and ^{186}Re .
121. An immunotoxin comprising an antibody recognizing VCAM1 or VCAM1b conjugated to a cell toxin.
122. The immunotoxin of claim 121 wherein the cell toxin is Pseudomonas exotoxin.
123. A method for detecting VCAM1 or VCAM1b-producing cancer cells comprising the step of administering a radioimmunoconjugate having an antibody which recognizes VCAM1 or VCAM1b.
124. A method for treating cancer comprising the step of administering an effective dose of a radioimmunoconjugate or immunotoxin having an antibody which recognizes VCAM1 or VCAM1b.
125. A DNA sequence encoding a VCAM/ICAM fusion protein comprising DNA sequences for VCAM1 or VCAM1b domains binding a VCAM1 ligand and ICAM1 domains binding an ICAM1 ligand.
126. A DNA sequence according to claim 125 wherein the VCAM1 ligand is VLA4 and the ICAM1 ligand is LFA1.
127. A VCAM/ICAM fusion protein comprising VCAM1 or VCAM1b domains binding a VCAM1 ligand and ICAM1 domains binding an ICAM1 ligand.
128. A fusion protein according to claim 127 wherein the VCAM1 ligand is VLA4 and the ICAM1 ligand is LFA1.
129. A method for treating tumors expressing ELAM1, VCAM1, VCAM1b or a ligand thereof comprising: (1) removing a sample of tumor tissue from a mammal having such a tumor, (2) isolating from said sample one or more leukocytes that have infiltrated the tumor tissue, (3) transfecting said one or more infiltrating leukocytes with a recombinant expression vector including a gene coding for a tumoricidal agent and capable of expressing in said leukocytes a tumoricidal gene product, (4) introducing the transfected leukocytes into said mammal.
130. A method according to claim 129, wherein the tumoricidal gene product is TNF or lymphotoxin.
131. A method according to claim 130, wherein the mammal is a human.
132. A method according to claim 129, wherein the recombinant expression vector is a retroviral vector.

the transfected leukocytes into the mammal, said transfected leukocytes are expanded with IL-2.

134. A method according to claim 129, wherein the tumor is a malignant tumor.

135. A method according to claim 129, wherein the tumor expresses VLA4 or CDX.

136. A method according to claim 135, wherein the tumor is melanoma or colon carcinoma.

137. A method for enhancing the cytolytic properties of leukocytes against target cells expressing ELAMs or MILAs comprising transfecting said leukocytes with a recombinant expression vector including a gene coding for an ELAM or MILA molecule that binds to the ELAM or MILA expressed by said target cells, which recombinant expression vector is capable of expressing said gene in said leukocyte.

138. A method according to 137, wherein the target cell is a melanoma or a colon carcinoma.

139. A method according to claim 138, wherein the gene encodes VCAM1 or VCAM1b, or encodes ELAMI.

140. A method according to claim 137, wherein the recombinant expression vector is a retroviral vector.

141. A method according to claim 140, wherein the recombinant expression vector also includes a gene encoding a tumoricidal agent.

142. A method according to claim 140, wherein the leukocytes are tumor infiltrating leukocytes.

143. A DNA sequence selected from the group consisting of: (a) the DNA sequence of FIG. 9 from nucleotide 66 to 1280; (b) the DNA sequence of FIG. 9 from nucleotide 69 to 1280; (c) the DNA sequence of FIG. 10 from nucleotide 172 to 1761; (d) the DNA sequence of FIG. 10 from nucleotide 175 to 1761; (e) DNA sequences that hybridize to any of the foregoing DNA sequences under standard hybridization conditions and have the biological activity of protein 7.2 or protein 1; and (f) DNA sequences that code on expression for an amino acid sequence encoded by any of the foregoing DNA sequences.

144. A recombinant DNA molecule comprising a DNA sequence selected from the group consisting of: (a) the DNA sequence of FIG. 9 from nucleotide 66 to 1280; (b) the DNA sequence of FIG. 9 from nucleotide 69 to 1280; (c) the DNA sequence of FIG. 10 from nucleotide 172 to 1761; (d) the DNA sequence of FIG. 10 from nucleotide 175 to 1761; (e) DNA sequences that hybridize to any of the foregoing DNA sequences under standard hybridization conditions and have the biological activity of protein 7.2 or protein 1; and (f) DNA sequences that code on expression for an amino acid sequence encoded by any of the foregoing DNA sequences.

145. The recombinant DNA molecule according to claim 144 wherein said DNA sequence is operatively linked to an expression control sequence.

146. A unicellular host transformed with a recombinant DNA molecule comprising a DNA sequence encoding an amino acid sequence of FIG. 9 or FIG. 10.

147. A unicellular host of claim 146 selected from the group consisting of E.coli, Pseudomonas, Bacillus, Streptomyces, yeasts, CHO, R1.1, B-W, L-M, COS 1, COS 7, BSC1, BSC40, BMT10, insect cells, plant cells, and human cells in tissue culture.

148. A protein produced by the method of expressing in a unicellular host a recombinant DNA molecule according to claim 144.

149. A process for producing a molecule that binds to ELAM1 comprising the step of expressing a DNA sequence encoding the amino acid sequence of FIG. 9 or FIG. 10 in a eukaryotic host cell.

150. A process for producing a cell that adheres to ELAM1 comprising the step of expressing a DNA sequence encoding the amino acid sequence of FIG. 9 or FIG. 10 in a eukaryotic host cell.

151. Pseudo-X or a fragment thereof capable of binding to α -CDX.

152. Pseudo-X₂ or a fragment thereof capable of binding to α -CDX.

moiety of a protein or a fucose-containing portion thereof wherein the protein is selected from the group consisting of CDX, Pseudo-X or Pseudo-X₂.

154. A molecule according to claim 153 wherein the protein is CDX.

155. A method of inhibiting adhesion between leukocytes and endothelial cells in a system containing them comprising the step of introducing in said system an effective amount of a molecule capable of binding to ELAM1, which molecule comprises a carbohydrate moiety of a protein or a fucose-containing portion thereof, wherein the protein is selected from the group consisting of CDX, Pseudo-X and Pseudo-X₂.

156. The method according to claim 155 wherein the protein is CDX.

157. A small molecule that inhibits the activity of the 1,3-fucosyl transferases described herein.

158. A method of identifying small molecules that inhibit the activity of the 1,3-fucosyl transferases described herein comprising the steps of: (1) contacting together an inhibitor candidate, a fucose acceptor and a 1,3-fucosyl transferase to create a test mixture and (2) assaying the test mixture for 1,3-fucosyl transferase activity.

159. The method according to claim 158 wherein the fucose acceptor is LacNAc.

160. The method according to claim 158 wherein the fucose acceptor 2'-fucosyllactose.

161. The method according to claim 158 wherein the 1,3-fucosyl transferase is derived from an extract from a cell transformed with clone 7.2 or clone 1.

162. The use of a 1,3-fucosyl transferase in a process for synthesizing an organic compound.

163. A method of producing a 1,3 glycosidic bond between fucose and a fucose acceptor comprising the step of catalysis with a 1,3-fucosyl transferase as described herein.

164. The method according to claim 163 in which the fucose acceptor is a carbohydrate.

165. Protein 7.2, protein 1, non-human homologues of protein 7.2 or protein 1 and biologically active fragments of any of the foregoing proteins.

166. A cytokine-inducible expression control sequence derived from the nucleotide sequence of FIG. 8.

167. The cytokine-inducible expression control sequence of claim 166 comprising nucleotides 210-408 of FIG. 8.

168. A ribozyme according to claim 98 that cleaves mRNA for clone 7.2 or clone 1.

L5 ANSWER 45 OF 112 USPATFULL on STN

2003:219652 Nucleic acid detection in pooled samples.

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US 2003152942 A1 20030814

APPLICATION: US 2002-142283 A1 20020509 (10)

PRIORITY: US 2001-326549P 20011002 (60)

US 2001-289764P 20010509 (60)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to detecting target nucleic acid sequences in pooled samples. In particular, the present invention relates to compositions and methods for detecting the presence or absence of target nucleic acid sequences (e.g. RNA virus sequences) in a pooled sample employing an INVADER detection assay. In certain embodiments, the present invention allows target nucleic acid sequence detection in pooled biological samples (e.g. pooled blood samples) without prior amplification of the target.

CLM What is claimed is:

1. A method of performing nucleic acid testing on a pooled sample, comprising: a) providing; i) a pooled sample, wherein said pooled

individual samples; and ii) INVADER assay reagents configured to detect the presence or absence of a target nucleic acid sequence; and b) contacting said pooled sample with said INVADER assay reagents under conditions such that the presence or absence of said target nucleic acid sequence in said pooled sample is determined.

2. The method of claim 1, wherein said biological material comprises blood.

3. The method of claim 1, wherein said biological material comprises blood plasma.

4. The method of claim 1, wherein said target nucleic acid sequence is RNA.

5. The method of claim 1, wherein said target nucleic acid sequence is DNA.

6. The method of claim 1, wherein said target nucleic acid sequence is from a microorganism.

7. The method of claim 1, wherein said target nucleic acid sequence is from a virus.

8. The method of claim 1, wherein said target nucleic acid sequences is from a pathogen selected from HIV-1, HIV-2, HCV, HBV, HTLV1, HTLV2, and HCMV.

9. The method of claim 1, wherein said target nucleic acid comprises a first and second non-contiguous single-stranded regions separated by an intervening region comprising a double stranded regions, and wherein said INVADER assay reagents comprise; i) a bridging **oligonucleotide** capable of binding to said first and second non-contiguous single-stranded regions; ii) a second **oligonucleotide** capable of binding to a portion of said first non-contiguous single-stranded region; and iii) a cleavage means.

10. The method of claim 9, wherein said contacting causes either said second **oligonucleotide** or said bridging **oligonucleotide** to be cleaved.

11. The method of claim 1, wherein said plurality of individual samples comprises at least 5 individual samples.

12. The method of claim 1, wherein said plurality of individual samples comprises at least 16 individual samples.

13. The method of claim 1, further comprising, prior to step b), the step of performing **polymerase chain reaction** on said pooled sample such that said target nucleic acid sequence is amplified if present in said pooled sample.

14. The method of claim 1, wherein said contacting step is performed under conditions such that said target nucleic acid sequence is not amplified before said presence or said absence of said target nucleic acid sequence is determined.

15. A method of performing nucleic acid testing on a pooled sample, comprising: a) providing; i) a plurality of individual biological material samples; and ii) INVADER assay reagents configured to detect the presence or absence of a target nucleic acid sequence; and b) forming a sub-pool by combining a portion of each of said plurality of individual biological samples, and c) contacting said sub-pool with said INVADER assay reagents under conditions such that the presence or absence of said target nucleic acid sequence in said sub-pool is determined.

16. The method of claim 15, wherein said contacting indicates that said target nucleic acid sequence is absent from said sub-pool.

17. The method of claim 16, further comprising the step of combining said plurality of individual biological samples into a primary pool.

18. The method of claim 15, wherein said contacting indicates that said target nucleic acid sequence is present in said sub-pool.

19. The method of claim 18, further comprising the step of screening each of said individual biological samples for the presence or absence of said target nucleic acid sequence.

20. A method of performing nucleic acid testing on a pooled sample, comprising: a) providing; i) a pooled sample, wherein said pooled sample comprises biological material combined from a plurality of

measure the quantity of a target nucleic acid sequence present in a sample; and b) contacting said pooled sample with said INVADER assay reagents under conditions such that the quantity of said target nucleic acid sequence present in said pooled sample is determined.

21. The method of claim 20, wherein said biological material comprises blood.

22. The method of claim 20, wherein said biological material comprises blood plasma.

23. The method of claim 20, wherein said target nucleic acid sequence is RNA.

24. The method of claim 20, wherein said target nucleic acid sequence is DNA.

25. The method of claim 20, wherein said target nucleic acid sequence is from a microorganism.

26. The method of claim 20, wherein said target nucleic acid sequence is from a virus.

27. A method for detecting an allele frequency of a polymorphism, comprising: a) providing; i) a pooled sample, wherein said pooled sample comprises target nucleic acid sequences from a plurality of individuals; and ii) INVADER assay reagents configured to detect the presence or absence of a polymorphism; and b) contacting said pooled sample with said INVADER assay reagents to generate a detectable signal; and c) measuring said detectable signal, thereby determining a number of said target nucleic acid sequences that contain said polymorphism.

28. The method of claim 27, wherein said plurality comprises at least 10 individuals.

29. The method of claim 28, wherein said at least 10 individuals comprises at least 1000 individuals.

30. A method for detecting an allele frequency of a polymorphism, comprising: a) providing; i) a pooled sample, wherein said pooled sample comprises target nucleic acid sequences from a plurality of individuals; and ii) INVADER assay reagents configured to generate distinct signals for each allele of a polymorphic locus in said target nucleic acid sequence; b) contacting said pooled sample with said INVADER assay reagents to generate at least one distinct signal; and c) measuring each of said at least one distinct signal, thereby determining a proportion of each allele of said polymorphic locus within said pooled sample.

31. The method of claim 30, wherein said measuring comprises detection of fluorescence.

32. The method of claim 30, wherein at least two distinct signals are generated in step b) and wherein said measuring comprises comparing said at least two distinct signals.

33. The method of claim 32, wherein said comparing comprises applying a correction factor to a measurement of at least one distinct signal.

34. A method for detecting a rare mutation comprising; a) providing; i) a sample from a single subject, wherein said sample comprises at least 10,000 target nucleic acid sequences, ii) a detection assay capable of detecting a mutation in a population of target nucleic acid sequence that is present at an allele frequency of 1:1000 or less compared to wild type alleles; and b) assaying said sample with said detection assay under conditions such that the presence or absence of a rare mutation is detected.

35. A method for detecting a rare mutation comprising; a) providing; i) a sample from a single subject, wherein said sample comprises at least 10,000 target nucleic acid sequences, ii) a detection assay capable of detecting a mutation in a population of target nucleic acid sequence that is present at an allele frequency of 1:1000 or less compared to wild type alleles; and b) assaying said sample with said detection assay under conditions such that an allele frequency in said sample of a rare mutation is determined.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

- AB The invention concerns GENSET polynucleotides and polypeptides. Such GENSET products may be used as reagents in forensic analyses, as chromosome markers, as tissue/cell/organelle-specific markers, in the production of expression vectors. In addition, they may be used in screening and diagnosis assays for abnormal GENSET expression and/or biological activity and for screening compounds that may be used in the treatment of GENSET-related disorders.
- CLM What is claimed is:
1. An isolated polynucleotide, said polynucleotide comprising a nucleic acid sequence encoding: i) a polypeptide comprising an amino acid sequence having any one of the sequences shown as SEQ ID NOs: 242-482 or any one of the sequences of polypeptides encoded by the clone inserts of the deposited clone pool; or ii) a biologically active fragment of said polypeptide.
 2. The polynucleotide of claim 1, wherein said polypeptide comprises any one of the sequences shown as SEQ ID NOs: 242-482 or any one of the sequences of the polypeptides encoded by the clone inserts of the deposited clone pool.
 3. The polynucleotide of claim 1, wherein said polypeptide comprises a signal peptide.
 4. The polynucleotide of claim 1, wherein said polypeptide is a mature protein.
 5. The polynucleotide of claim 1, wherein said nucleic acid sequence has at least about 100 contiguous nucleotides of any one of the sequences shown as SEQ ID NOs: 1-241 or any one of the sequences of the clone inserts of the deposited clone pool.
 6. The polynucleotide of claim 1, wherein said polynucleotide is operably linked to a promoter.
 7. An expression vector comprising the polynucleotide of claim 6.
 8. A host cell recombinant for the polynucleotide of claim 1.
 9. A non-human transgenic animal comprising the host cell of claim 8.
 10. A method of making a GENSET polypeptide, said method comprising a) providing a population of host cells comprising the polynucleotide of claim 6; and b) culturing said population of host cells under conditions conducive to the production of said polypeptide within said host cells.
 11. The method of claim 10, further comprising purifying said polypeptide from said population of host cells.
 12. A method of making a GENSET polypeptide, said method comprising a) providing a population of cells comprising the polynucleotide of claim 6; b) culturing said population of cells under conditions conducive to the production of said polypeptide within said cells; and c) purifying said polypeptide from said population of cells.
 13. An isolated polypeptide or biologically active fragment thereof, said polypeptide comprising an amino acid sequence having any one of the sequences shown as SEQ ID NOs: 242-482 or any one of the sequences of polypeptides encoded by the clone inserts of the deposited clone pool.
 14. The polypeptide of claim 13, wherein said polypeptide comprises a signal peptide.
 15. The polypeptide of claim 13, wherein said polypeptide is a mature protein.
 16. An antibody that specifically binds to the polypeptide of claim 13.
 17. A method of binding the antibody of claim 16 with the polypeptide of claim 13 comprising the step of: contacting said antibody with said polypeptide under conditions that allow binding of said antibody to said protein.
 18. A method of determining whether a GENSET gene is expressed within a mammal, said method comprising the steps of: a) providing a biological sample from said mammal b) contacting said biological sample with

conditions to the polynucleotide of claim 1; or ii) a polypeptide that specifically binds to the polypeptide of claim 19; and c) detecting the presence or absence of hybridization between said polynucleotide and an RNA species within said sample, or the presence or absence of binding of said polypeptide to a protein within said sample; wherein a detection of said hybridization or of said binding indicates that said GENSET gene is expressed within said mammal.

19. The method of claim 18, wherein said polynucleotide is a **primer**, and wherein said hybridization is detected by detecting the presence of an amplification product comprising the sequence of said **primer**.

20. The method of claim 18, wherein said polypeptide is an antibody.

21. A method of determining whether a mammal has an elevated or reduced level of GENSET gene expression, said method comprising the steps of: a) providing a biological sample from said mammal; and b) comparing the amount of the polypeptide of claim 13, or of an RNA species encoding said polypeptide, within said biological sample with a level detected in or expected from a control sample; wherein an increased amount of said polypeptide or said RNA species within said biological sample compared to said level detected in or expected from said control sample indicates that said mammal has an elevated level of said GENSET gene expression, and wherein a decreased amount of said polypeptide or said RNA species within said biological sample compared to said level detected in or expected from said control sample indicates that said mammal has a reduced level of said GENSET gene expression.

22. A method of identifying a candidate modulator of a GENSET polypeptide, said method comprising: a) contacting the polypeptide of claim 19 with a test compound; and b) determining whether said compound specifically binds to said polypeptide; wherein a detection that said compound specifically binds to said polypeptide indicates that said compound is a candidate modulator of said GENSET polypeptide.

L5 ANSWER 47 OF 112 USPATFULL on STN

2003:219622 Multiple viral replicon culture systems.

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US 2003152912 A1 20030814

APPLICATION: US 2002-60941 A1 20020129 (10)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Methods and compositions are provided for screening candidate antiviral agents using cells containing subgenomic viral replication systems such as replicons and minigenomes. The methods involve the simultaneous assay of more than one subgenomic viral replication system. Compositions useful for these methods are also provided.

CLM What is claimed is:

1. A method of screening a candidate antiviral agent for antiviral activity comprising (a) preparing a first cell culture comprising cells containing a first subgenomic viral replication system, and a second cell culture comprising cells containing a second subgenomic viral replication system; (b) adding the candidate antiviral agent to each cell culture; (c) incubating the cell cultures under conditions and for a time sufficient to detect an antiviral effect by the candidate antiviral agent on the subgenomic viral replication systems; and (d) determining the effect of the candidate antiviral agent on each viral replication system, wherein the first subgenomic viral replication system is genetically distinct from the second subgenomic viral replication system.

2. The method of claim 1, wherein the first and second cell cultures are combined before step (b).

3. The method of claim 1, further comprising a cell culture not containing a subgenomic viral replication system.

4. The method of claim 1, wherein at least one of the subgenomic viral replication systems is a replicon.

5. The method of claim 1, wherein at least one of the subgenomic viral replication systems is a defective genome.

6. The method of claim 1, wherein the cells in the first and second cell cultures are mammalian cells and the first and second subgenomic viral replication systems are from mammalian viruses.

7. The method of claim 6, wherein the mammalian cells are human cells

8. The method of claim 7, wherein the human viruses are selected from the group consisting of hepatitis C virus, yellow fever virus, respiratory syncytia virus, Sindbis virus, poliovirus, Japanese encephalitis virus, hepatitis B virus, human papilloma virus, herpes simplex virus type 1, Epstein-Barr virus, adeno-associated virus, Venezuelan encephalitis virus, rubella, coxsackivirus, enterovirus, hepatitis A virus, Dengue fever virus, West Nile virus, tick-borne encephalitis virus, astrovirus, rabies virus, influenza virus A, influenza virus B, respiratory syncytial virus, measles, mumps, Ebola virus, Marburg virus, La Crosse virus, California encephalitis virus, Hantaan virus, Crimean-Congo virus, Rift Valley fever, Lassa fever, Argentine hemorrhagic fever virus, Bolivian hemorrhagic fever virus, Colorado tick fever, JC virus, BK virus, herpes simplex virus type two, human cytomegalovirus, varicella-zoster virus, human herpes simplex virus type six, human herpes virus type seven, human herpes virus type eight, human adenovirus, HIV-1, HIV-2, HTLV-1, HTLV-2, and human parvovirus.

9. The method of claim 7, wherein the human viruses are selected from the group consisting of hepatitis C virus, yellow fever virus, respiratory syncytia virus, Sindbis virus, poliovirus, Japanese encephalitis virus, hepatitis B virus, human papilloma virus, herpes simplex virus type 1, Epstein-Barr virus, and adeno-associated virus.

10. The method of claim 7, wherein the human viruses are selected from the group consisting of hepatitis C virus, respiratory syncytia virus, yellow fever virus and Sindbis virus.

11. The method of claim 1, wherein the candidate antiviral agent is a chemical that does not comprise an oligopeptide or an **oligonucleotide**.

12. The method of claim 1, wherein the candidate antiviral agent comprises an oligopeptide or an **oligonucleotide**.

13. The method of claim 1, wherein the candidate antiviral agent comprises an **oligonucleotide** or a polynucleotide.

14. The method of claim 1, wherein the candidate antiviral agent comprises a protein.

15. The method of claim 14, wherein the candidate antiviral agent comprises an antibody binding domain.

16. The method of claim 1, wherein the effect of the candidate antiviral agent on at least one of the subgenomic viral replication systems is determined by quantitation of a portion of the nucleic acid of the at least one subgenomic viral replication system.

17. The method of claim 16, wherein the quantitation is performed by nucleic acid amplification.

18. The method of claim 17, wherein the nucleic acid amplification is by RT-PCR.

19. The method of claim 1, wherein the effect of the antiviral agent on at least one of the subgenomic viral replication systems is determined by quantitation of a reporter gene or assayable portion of a fusion protein that is transcribed along with other viral proteins.

20. The method of claim 1, wherein the effect of the antiviral agent on at least one of the subgenomic viral replication systems is determined by quantitation of a viral protein.

21. The method of claim 20, wherein the viral protein is an enzyme and the quantitation is by assay of the activity of the enzyme.

22. The method of claim 1, wherein at least one of the cell cultures comprises cells wherein the subgenomic viral replication system is stably maintained.

23. The method of claim 1, wherein at least one of the cell cultures comprises cells wherein the subgenomic viral replication system is not stably maintained.

24. The method of claim 1, wherein at least one of the cell cultures comprises primary cells.

25. The method of claim 1, wherein the cell cultures are incubated at least 20 h.

26. The method of claim 1, further comprising at least a third cell

system, wherein the third cell culture is also subjected to steps (a), (b), (c) and (d), wherein the each subgenomic viral replication system is genetically distinct from every other subgenomic viral replication system.

27. The method of claim 26, wherein all cell cultures comprising a subgenomic viral replication system are combined before step (b).

28. A method of screening a candidate antiviral agent for antiviral activity comprising (a) combining a first cell culture comprising cells containing a first subgenomic viral replication system and a second cell culture comprising cells containing a second subgenomic viral replication system to make a mixed cell culture; (b) adding the candidate antiviral agent to the mixed cell culture; (c) incubating the mixed cell culture under conditions and for a time sufficient to detect an antiviral effect by the candidate antiviral agent on the subgenomic viral replication systems; and (d) determining the effect of the candidate antiviral agent on each viral replication system, wherein the first subgenomic viral replication system is genetically distinct from the second subgenomic viral replication system.

29. The method of claim 28, wherein at least one of the subgenomic viral replication systems is a replicon.

30. The method of claim 28, wherein at least one of the subgenomic viral replication systems is a defective genome.

31. The method of claim 28, wherein all of the cells of the mixed cell culture are the same cell line.

32. The method of claim 28, wherein the cells of the mixed cell culture comprise more than one cell line.

33. The method of claim 28, wherein all of the cells in the mixed cell culture are mammalian cells and all of the subgenomic viral replication systems are from mammalian viruses.

34. The method of claim 33, wherein the mammalian cells are human cells and the mammalian viruses are human viruses.

35. The method of claim 34, wherein the human viruses are selected from the group consisting of hepatitis C virus, respiratory syncytia virus, yellow fever virus, Sindbis virus, poliovirus, Japanese encephalitis virus, hepatitis B virus, human papilloma virus, herpes simplex virus type 1, Epstein-Barr virus, adeno-associated virus, Venezuela encephalitis virus, rubella, coxsackivirus, enterovirus, hepatitis A virus, Dengue fever virus, West Nile virus, tick-borne encephalitis virus, astrovirus, rabies virus, influenza virus A, influenza virus B, respiratory syncytial virus, measles, mumps, Ebola virus, Marburg virus, La Crosse virus, California encephalitis virus, Hantaan virus, Crimean-Congo virus, Rift Valley fever, Lassa fever, Argentine hemorrhagic fever virus, Bolivian hemorrhagic fever virus, Colorado tick fever, JC virus, BK virus, herpes simplex virus type two, human cytomegalovirus, varicella-zoster virus, human herpes simplex virus type six, human herpes virus type seven, human herpes virus type eight, human adenovirus, HIV-1, HIV-2, HTLV-1, HTLV-2, and human parvovirus.

36. The method of claim 34, wherein the human viruses are selected from the group consisting of hepatitis C virus, respiratory syncytia virus, yellow fever virus, Sindbis virus, poliovirus, Japanese encephalitis virus, hepatitis B virus, human papilloma virus, herpes simplex virus type 1, Epstein-Barr virus, and adeno-associated virus.

37. The method of claim 34, wherein the human viruses are selected from the group consisting of hepatitis C virus, respiratory syncytia virus, yellow fever virus and Sindbis virus.

38. The method of claim 28, wherein the candidate antiviral agent is a chemical that does not comprise an oligopeptide or an **oligonucleotide**.

39. The method of claim 28, wherein the antiviral agent comprises an oligopeptide or an **oligonucleotide**.

40. The method of claim 28, wherein the candidate antiviral agent comprises an **oligonucleotide** or a polynucleotide.

41. The method of claim 28, wherein the candidate antiviral agent comprises a protein.

42. The method of claim 41, wherein the candidate antiviral agent comprises an antibody binding domain.

antiviral agent on at least one of the subgenomic viral replication systems is determined by quantitation of a portion of the nucleic acid of the at least one subgenomic viral replication system.

44. The method of claim 43, wherein the quantitation is performed by nucleic acid amplification.

45. The method of claim 44, wherein the nucleic acid amplification is by RT-PCR.

46. The method of claim 28, wherein the effect of the antiviral agent on at least one of the subgenomic viral replication systems is determined by quantitation of a reporter gene or assayable portion of a fusion protein that is transcribed along with other viral proteins.

47. The method of claim 28, wherein the effect of the antiviral agent on at least one of the subgenomic viral replication systems is determined by quantitation of a viral protein.

48. The method of claim 47, wherein the viral protein is an enzyme and the quantitation is by assay of the activity of the enzyme.

49. The method of claim 28, wherein the mixed cell culture comprises cells wherein the subgenomic viral replication system is stably maintained.

50. The method of claim 28, wherein the mixed cell culture comprises cells wherein the subgenomic viral replication system is not stably maintained.

51. The method of claim 28, wherein the mixed cell culture comprises primary cells.

52. The method of claim 28, wherein the mixed cell culture is incubated at least 20 h.

53. The method of claim 28, wherein the mixed cell culture further comprises a third cell culture comprising cells containing a third subgenomic viral replication system.

54. The method of claim 53, wherein the mixed cell culture further comprises a fourth cell culture comprising cells containing a fourth subgenomic viral replication system.

55. A mixed cell culture comprising a first cell culture comprising cells containing a first subgenomic viral replication system and a second cell culture comprising cells containing a second subgenomic viral replication system.

56. The mixed cell culture of claim 55, wherein at least one of the subgenomic viral replication systems is a replicon.

57. The mixed cell culture of claim 55, wherein at least one of the subgenomic viral replication systems is a defective genome.

58. The mixed cell culture of claim 55, wherein all of the cells of the mixed cell culture are the same cell line.

59. The mixed cell culture of claim 55, wherein the cells of the mixed cell culture comprise more than one cell line.

60. The mixed cell culture of claim 55, wherein all of the cells in the mixed cell culture are mammalian cells and all of the subgenomic viral replication systems are from mammalian viruses.

61. The mixed cell culture of claim 60, wherein the mammalian cells are human cells and the mammalian viruses are human viruses.

62. The mixed cell culture of claim 61, wherein the human viruses are selected from the group consisting of hepatitis C virus, respiratory syncytia virus, yellow fever virus, Sindbis virus, poliovirus, Japanese encephalitis virus, hepatitis B virus, human papilloma virus, herpes simplex virus type 1, Epstein-Barr virus, adeno-associated virus, Venezuela encephalitis virus, rubella, coxsackivirus, enterovirus, hepatitis A virus, Dengue fever virus, West Nile virus, tick-borne encephalitis virus, astrovirus, rabies virus, influenza virus A, influenza virus B, respiratory syncytial virus, measles, mumps, Ebola virus, Marburg virus, La Crosse virus, California encephalitis virus, Hantaan virus, Crimean-Congo virus, Rift Valley fever, Lassa fever, Argentine hemorrhagic fever virus, Bolivian hemorrhagic fever virus, Colorado tick fever, JC virus, BK virus, herpes simplex virus type two, human cytomegalovirus, varicella-zoster virus, human herpes simplex

eight, human adenovirus, HIV-1, HIV-2, HTLV-1, HTLV-2, and human parvovirus.

63. The mixed cell culture of claim 61, wherein the human viruses are selected from the group consisting of hepatitis C virus, respiratory syncytia virus, yellow fever virus, Sindbis virus, poliovirus, Japanese encephalitis virus, hepatitis B virus, human papilloma virus, herpes simplex virus type 1, Epstein-Barr virus, and adeno-associated virus.

64. The mixed cell culture of claim 61, wherein the human viruses are selected from the group consisting of hepatitis C virus, respiratory syncytia virus, yellow fever virus and Sindbis virus.

65. The mixed cell culture of claim 55, wherein the mixed cell culture comprises cells wherein the subgenomic viral replication system is stably maintained.

66. The mixed cell culture of claim 55, wherein the mixed cell culture comprises cells wherein the subgenomic viral replication system is not stably maintained.

67. The mixed cell culture of claim 55, wherein the mixed cell culture comprises primary cells.

68. The mixed cell culture of claim 55, further comprising a third cell culture comprising cells containing a third subgenomic viral replication system.

69. The mixed cell culture of claim 68, wherein the mixed cell culture further comprises a fourth cell culture comprising cells containing a fourth subgenomic viral replication system.

L5 ANSWER 48 OF 112 USPATFULL on STN
2003:200834 Method for detecting disease.

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US 2003138819 A1 20030724

APPLICATION: US 2002-279628 A1 20021024 (10)

PRIORITY: US 2001-335875P 20011026 (60)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to diagnostic methods utilizing an apparatus comprising a substrate having at least one assay station. The at least one assay station has at least a first assay station channel and at least a second assay station channel and the first and second assay station channels each separately being in communication with the at least one assay station. The apparatus has an arrangement of at least first and second multi-purpose channels in communication with the first and second assay station channels, respectively. The first multipurpose channel and first assay station channel have internal surface characteristics conducive to conduction of a sample solution therethrough. There is at least one sample fluid inlet in communication with the at least first multi-purpose channel, and at least one isolation-medium inlet in communication with the at least first and second multi-purpose channels. The at least one second multi-purpose channel has an internal surface portion non-conductive to conduction of said sample solution.

CLM What is claimed is:

1) A method for diagnosing and analyzing biological samples comprising: providing a substrate having at least one assay station, an arrangement of at least first and second multi-purpose channels wherein said at least one assay station being situated in a position intermediate between said first and second multipurpose channels and in fluid communication therewith, wherein said first multi-purpose channel has at least one characteristic conducive to conduction of a sample fluid therethrough, at least one sample fluid inlet in communication with said at least first multi-purpose channel; and at least one isolation-medium inlet in communication with said at least first and second multi-purpose channels, said at least one second multi-purpose channel having at least one characteristic non-conductive to conduction of said sample fluid; obtaining a test sample from a subject; preparing from said test sample a sample fluid; introducing a sample fluid to at least one sample fluid inlet; filling said at least one assay station via said at least one multi-purpose channel; allowing isolation-medium from said at least one isolation medium port to flow into at least said first multi-purpose channel; and running at least one reaction at said at least one assay station, said reaction providing at least one of qualitative or quantitative data relating to said sample fluid.

2) The method according to claim 1 wherein said at least one reaction is

- 3) The method according to claim 2 wherein said homogenous assay reaction is at least one of a nucleic acid based assay, a protein/antibody assay and cell based assay.
- 4) The method according to claim 3 wherein said nucleic acid based assay is at least one of a **polymerase chain reaction** or a reverse-transcriptase **polymerase chain reaction**.
- 5) The method according to claim 1 further comprising the step of at least one of homogenizing, digesting and filtering said test sample before injection into said sample fluid inlet.
- 6) The method according to claim 1 further comprising the step of applying a sealing layer to seal said at least one assay station.
- 7) The method according to claim 6 further comprising placing within said at least one assay station at least one component of said at least one reaction.
- 8) The method according to claim 7 further comprising a drying or lyophilization step after said placing step.
- 9) The method according to claim 7 wherein said at least one component of said at least one reaction is at least one of a labeled probe or marker.
- 10) The method according to claim 1, wherein said fluid communication is via at least first and second assay station channels in communication with said first and second multipurpose channels.
- 11) The method according to claim 2 wherein said homogenous assay reaction provides for detection of a nucleic acid sequence associated with the presence of a pathogen.
- 12) The method according to claim 1 wherein said at least one of qualitative or quantitative data is provided by at least one of fluorescence resonance energy transfer, luminescence or calorimetric change.
- 13) The method according to claim 2 further comprising the step of irradiating contents of said at least one assay station after running at least one reaction or at least a portion of said at least one reaction.
- 14) The method according to claim 7 wherein said at least one component of said at least one reaction is at least one of an antibody, protein and at least one **primer**.
- 15) The method according to claim 7 wherein said at least one component of said at least one reaction is at least one of a synthetic molecule from a combinatorial library of molecules, a peptide library and an aptamer library.
- 16) The method according to claim 15 further comprising the step of introducing at least one of a population of wild-type cells and a population of cells expressing a recombinant molecule, into said at least one assay station.
- 17) The method according to claim 1 wherein said at least one of qualitative or quantitative data is provided by quenching or unquenching of a fluorescent label.
- 18) The method according to claim 12 wherein said fluorescence resonance energy transfer is provided by protein-protein interactions wherein a first protein component of said protein-protein interactions is immobilized in said assay station and a second protein component of said protein-protein interaction is introduced into said assay station, where said interaction occurs upon association of said first and second of said protein component s such that said energy transfer may take place.
- 19) The method according to claim 1 wherein said first multipurpose channel characteristic conducive to conduction of said sample fluid comprises at least one of internal surface characteristic and/or shape characteristic and said at least one second multipurpose channel characteristic that is non-conductive to conduction of said sample fluid comprises at least one of an internal surface portion and/or shape characteristics.
- 20) The method according to claim 1 further comprising a sealing step wherein exposed portions of the said at least first and second multipurpose channels are sealed with a solid from ambient atmosphere adhesively, mechanically, electrically, or magnetically after the first

an isolation medium.

21) A method for diagnosing and analyzing biological samples comprising: providing a substrate having at least one assay station, an arrangement of at least first and second multi-purpose channels wherein said at least one assay station being situated in a position intermediate between said first and second multipurpose channels and in fluid communication therewith, wherein said first multi-purpose channel has at least one characteristic conducive to conduction of a sample fluid therethrough, at least one sample fluid inlet in communication with said at least first multi-purpose channel, and at least one isolation-medium inlet in communication with said at least first and second multi-purpose channels, said at least one second multi-purpose channel having at least one characteristic non-conductive to conduction of said sample fluid; introducing a sample fluid to at least one sample fluid inlet; filling said at least one assay station via said at least one multi-purpose channel; allowing isolation-medium from said at least one isolation medium port to flow into at least said first multi-purpose channel; and running at least one reaction at said at least one assay station, said reaction providing at least one of qualitative or quantitative data relating to said sample fluid.

22) The method according to claim 21 further comprising the step of obtaining a test sample from a subject.

23) The method according to claim 22 further comprising the step of preparing from said test sample a sample fluid.

24) The method according to claim 21 wherein said at least one reaction is a homogenous assay reaction.

25) The method according to claim 21 wherein said reaction is at least one of a nucleic acid based assay, a protein/antibody assay and cell based assay.

26) The method according to claim 25 wherein said nucleic acid based assay includes a nucleic acid amplification reaction.

27) The method according to claim 25 wherein said nucleic acid based assay is a hybridization assay having at least one nucleic acid derived probe.

28) The method according to claim 27 wherein said nucleic acid-derived probe is labeled with fluorescent dye.

29) The method according to claim 25 wherein said protein/antibody assay is an ELISA-based assay.

30) The method according to claim 26 wherein said nucleic acid amplification reaction is at least one of a **polymerase chain reaction**, a reverse-transcriptase **polymerase chain reaction** and isothermal amplification reaction.

31) The method according to claim 23 wherein said preparing step further comprising the step of at least one of homogenizing, digesting, purifying, sorting, concentrating and filtering said test sample before injection into said sample fluid inlet.

32) The method according to claim 21 wherein said providing step further comprises a step of applying a sealing layer to said apparatus to seal said at least one assay station.

33) The method according to claim 32 further comprising the step of placing within said at least one assay station at least one component of said at least one reaction before said sealing layer application step.

34) The method according to claim 33 further comprising a drying or lyophilization step after said placing step.

35) The method according to claim 34 further comprising an immobilizing step for immobilizing said least one component onto at least one of a surface of said assay station, beads, gels and membranes.

36) The method according to claim 33 wherein said at least one component of said at least one reaction is at least one of a labeled probe, ligand and reaction substrate.

37) The method according to claim 21 wherein said fluid communication is via at least first and second assay station channels in communication with said first and second multipurpose channels.

38) The method according to claim 21 wherein said reaction provides for

pathogen.

39) The method according to claim 38 wherein said pathogen is a microbial organism.

40) The method according to claim 38 wherein said pathogen is a virus, bacterium, fungus or protozoan.

41) The method according to claim 21 wherein said at least one of qualitative or quantitative data is provided by at least one of fluorescence resonance energy transfer, fluorescence quenching, fluorescence polarization, bioluminescence resonance energy transfer or beta-gal complementation assay.

42) The method according to claim 21 further comprising the step of irradiating contents of said at least one assay station after running at least one reaction or at least a portion of said at least one reaction.

43) The method according to claim 33 wherein said at least one component of said at least one reaction is at least one of an antibody, protein, at least one **primer**, nucleic acid, peptide, protein, drug, or small molecule.

44) The method according to claim 33 wherein said at least one component of said at least one reaction is at least one of a synthetic molecule from a combinatorial library of molecules, a peptide library, a nucleic acid library and an aptamer library.

45) The method according to claim 21 wherein said reaction provides for screening of potential drug candidates.

46) The method according to claim 33 further comprising the step of introducing at least one of a population of wild-type cells and a population of cells expressing a recombinant molecule into said at least one assay station.

47) The method according to claim 41 wherein said fluorescence resonance energy transfer is provided by protein-protein interactions wherein a first protein component of said protein-protein interactions is immobilized in said assay station and a second protein component of said protein-protein interaction is introduced into said assay station, where said interaction occurs upon association of said first and second of said protein components such that said energy transfer may take place.

48) The method according to claim 21 wherein said first multipurpose channel characteristic conducive to conduction of said sample fluid comprises at least one of internal surface characteristic and/or shape characteristic and said at least one second multipurpose channel characteristic that is non-conductive to conduction of said sample fluid comprises at least one of an internal surface portion and/or shape characteristics.

49) The method according to claim 21 further comprising a sealing step wherein exposed portions of the said at least first and second multipurpose channels are sealed with a solid from ambient atmosphere adhesively, mechanically, electrically, or magnetically after the first and second multipurpose channels are filled with a sample fluid and/or an isolation medium.

50) The method according to claim 21 further comprising a washing step in order to wash away at least one undesired reaction component.

51) The method according to claim 21 wherein said reaction provides for the detection of a variation in nucleic acid sequence associated with at least one of virulence, disease, phenotype, interindividual or interspecific differences.

52) The method according to claim 51 wherein said variation in nucleic acid sequence includes at least one of single nucleotide polymorphism, tandem repeats and insertions and/or deletions.

L5 ANSWER 49 OF 112 USPATFULL on STN

2003:200469 Microparticles for delivery of heterologous nucleic acids.

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CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Microparticles with adsorbent surfaces, methods of making such microparticles, and uses thereof, are disclosed. The microparticles comprise a polymer, such as a poly(α -hydroxy acid), a polyhydroxy butyric acid, a polycaprolactone, a polyorthoester, a polyanhydride, and the like, and are formed using cationic, anionic, or nonionic detergents. Also provided are microparticles in the form of submicron emulsions of an oil droplet emulsion having a metabolizable oil and an emulsifying agent. The surface of the microparticles efficiently adsorb polypeptides, such as antigens, and nucleic acids, such as ELVIS vectors and other vector constructs, containing heterologous nucleotide sequences encoding biologically active macromolecules, such as polypeptides, antigens, and adjuvants. Methods of stimulating an immune response, methods of immunizing a host animal against a viral, bacterial, or parasitic infection, and uses of the microparticle compositions for vaccines are also provided.

CLM What is claimed is:

1. A method of raising an immune response in a host animal comprising: administering to the animal a vector construct comprising a heterologous nucleic acid sequence encoding a first antigen in an amount effective to elicit an immunological response, wherein the vector construct is adsorbed onto microparticles comprising (i) a polymer selected from the group consisting of a poly(α -hydroxy acid), a polyhydroxy butyric acid, a polycaprolactone, a polyorthoester, a polyanhydride and a polycyanoacrylate and (ii) a detergent; and subsequently boosting the immunological response by administering a second antigen to the animal, wherein the first antigen and the second antigen can be the same or different.

2. The method of claim 1, wherein the first antigen and the second antigen are the same.

3. The method of claim 1, wherein the vector construct is selected from a plasmid DNA and an RNA vector construct.

4. The method of claim 3, wherein the plasmid DNA is an ELVIS vector.

5. The method of claim 4, wherein the ELVIS vector comprises a cDNA complement of an RNA vector construct derived from a member selected from the group consisting of alphavirus, picornavirus, togavirus, flavivirus, coronavirus, paramyxovirus, and yellow fever virus.

6. The method of claim 5, wherein the alphavirus is selected from the group consisting of Sindbis virus, Semliki Forest virus, Venezuelan equine encephalitis virus, or Ross River virus.

7. The method of claim 3, wherein the plasmid DNA comprises a CMV promoter/enhancer.

8. The method of claim 1, wherein the first and second antigens are selected from the group consisting of HIV antigens, hepatitis C virus antigens, and influenza A virus antigens.

9. The method of claim 1, wherein the first and second antigens comprise antigens selected from the group consisting of HIV antigens gp120, gp140, gp160, p24gag and p55gag.

10. The method of claim 1, wherein the first and second antigens comprise HIV p55gag.

11. The method of claim 1, wherein the first and second antigens comprise HIV gp140.

12. The method of claim 1, wherein the second antigen is adsorbed to microparticles comprising (i) a polymer selected from the group consisting of a poly(α -hydroxy acid), a polyhydroxy butyric acid, a polycaprolactone, a polyorthoester, a polyanhydride, and a polycyanoacrylate and (ii) a detergent.

13. The method of claim 1, wherein the second antigen is coadministered with an adjuvant.

14. The method of claim 13, wherein the adjuvant is MF59.

15. The method of claim 1, wherein the polymer comprises a poly(α -hydroxy acid) selected from the group consisting of poly(L-lactide), poly(D,L-lactide) and poly(D,L-lactide-co-glycolide)

CTAB, benzalkonium chloride, DDA and DOTAP.

16. The method of claim 1, wherein the vector construct is administered two or more times before the second antigen is administered.

17. The method of claim 16, wherein the second antigen is also administered two or more times.

18. The method of claim 17, wherein the vector construct is administered (a) at a time of initial administration, (b) at a time period ranging 1-8 weeks from the initial administration, and (c) at a time period ranging 4-32 weeks from the initial administration, and wherein the second antigen is administered (a) at a time period ranging from 8-50 weeks from the initial administration and (b) at a time period ranging from 8-100 weeks from the initial administration.

19. The method of claim 1, wherein the animal is a mammal selected from rhesus macaque and a human.

20. The method of claim 1, wherein the vector construct and the second antigen are administered subcutaneously, intraperitoneally, intradermally, intravenously or intramuscularly.

21. The method of claim 20, wherein the vector construct and the second antigen are administered intramuscularly.

22. The method of claim 1, wherein the vector construct is coadministered with an adjuvant.

23. The method of claim 1, wherein said immune response comprises a Th1 immune response.

24. The method of claim 1, wherein said immune response comprises a CTL immune response.

25. The method of claim 1, wherein said immune response is raised against a viral, bacterial, or parasitic infection.

26. A microparticle with an adsorbent surface to which a first biologically active macromolecule has been adsorbed comprising: a microparticle selected from the group consisting of (a) a polymer microparticle comprising: (i) a polymer selected from the group consisting of a poly(α -hydroxy acid), a polyhydroxy butyric acid, a polycaprolactone, a polyorthoester, a polyanhydride, and a polycyanoacrylate; and a detergent; and (b) a submicron emulsion comprising: (i) a metabolizable oil; and (ii) one or more emulsifying agents; and the first biologically active macromolecule, wherein the first biologically active macromolecule is a nucleic acid molecule comprising at least one vector construct selected from the group consisting of an ELVIS vector and an RNA vector construct.

27. The microparticle of claim 26, wherein said submicron emulsion is selected as said microparticle.

28. The microparticle of claim 26, wherein said polymer microparticle is selected as said microparticle.

29. The microparticle of claim 28, wherein the polymer microparticle comprises a poly(α -hydroxy acid) selected from the group consisting of poly(L-lactide), poly(D,L-lactide) and poly(D,L-lactide-co-glycolide).

30. The microparticle of claim 28, wherein the polymer comprises poly(D,L-lactide-co-glycolide).

31. The microparticle of claim 28, further comprising a second biologically active macromolecule entrapped within the microparticle, wherein the second biologically active macromolecule is a member selected from the group consisting of a polynucleotide, a polynucleoside, a pharmaceutical, a polypeptide, a hormone, an enzyme, a transcription or translation mediator, an intermediate in a metabolic pathway, an immunomodulator, an antigen, and an adjuvant.

32. The microparticle of claim 28, wherein said vector construct is an ELVIS vector.

33. The microparticle of claim 28, wherein said vector construct is an ELVIS vector comprising a cDNA complement of an RNA vector construct derived from a member selected from the group consisting of alphavirus, picornavirus, togavirus, flavivirus, coronavirus, paramyxovirus, and yellow fever virus, and wherein said RNA vector construct further comprises a selected heterologous nucleotide sequence.

34. The microparticle of claim 33, wherein said ELVIS vector is derived from an alphavirus selected from the group consisting of Sindbis virus, Semliki Forest virus, Venezuelan equine encephalitis virus, or Ross River virus.

35. The microparticle of claim 28, wherein said vector construct is an RNA vector construct derived from a member selected from the group consisting of alphavirus, picornavirus, togavirus, flavivirus, coronavirus, paramyxovirus, and yellow fever virus, and wherein said RNA vector construct comprises a selected heterologous nucleotide sequence.

36. The microparticle of claim 35, wherein said RNA vector construct is derived from an alphavirus selected from the group consisting of Sindbis virus, Semliki Forest virus, Venezuelan equine encephalitis virus, or Ross River virus.

37. The microparticle of claim 32, wherein said vector construct comprises a heterologous nucleic acid sequence encoding a member selected from the group consisting of a pharmaceutical, a polypeptide, a hormone, an enzyme, a transcription or translation mediator, an intermediate in a metabolic pathway, an immunomodulator, an antigen, and an adjuvant.

38. The microparticle of claim 37, wherein said heterologous nucleic acid sequence encodes an antigen.

39. The microparticle of claim 38, wherein said antigen is a member selected from the group consisting of HIV gp120, HIV gp140, HIV p24gag, HIV p55gag, and Influenza A hemagglutinin antigen.

40. The microparticle of claim 32, wherein said vector construct is a vector selected from the group consisting of the ELVIS vectors pSINCP-gp140 and pSINCP-p55gag.

41. The microparticle of claim 28, further comprising at least one second biologically active macromolecule adsorbed on the surface thereof, wherein the second biologically active macromolecule is at least one member selected from the group consisting of a polypeptide, a polynucleotide, a polynucleoside, an antigen, a pharmaceutical, a hormone, an enzyme, a transcription or translation mediator, an intermediate in a metabolic pathway, an immunomodulator, and an adjuvant.

42. The microparticle of claim 41, wherein the second biologically active macromolecule is an antigen.

43. The microparticle of claim 42, wherein the second biologically active macromolecule is an antigen selected from the group consisting of HIV gp120, HIV gp140, HIV p24gag, HIV p55 gag, and Influenza A hemagglutinin antigen.

44. The microparticle of claim 41, wherein the second biologically active macromolecule is a polynucleotide which encodes HIV gp140.

45. The microparticle of claim 41, wherein the second biologically active macromolecule is an adjuvant.

46. The microparticle of claim 45, wherein the adjuvant is an aluminum salt.

47. A microparticle composition comprising a microparticle of claim 26 and a pharmaceutically acceptable excipient.

48. The microparticle composition of claim 47, further comprising an adjuvant.

49. The microparticle composition of claim 48, wherein the adjuvant is a member selected from the group consisting of a CpG **oligonucleotide**.

50. The microparticle composition of claim 48, wherein the adjuvant is an aluminum salt which is aluminum phosphate.

51. A method of producing a microparticle having an adsorbent surface to which a vector construct capable of expressing a selected nucleic acid sequence is adsorbed, said method comprising the steps of: (a) emulsifying a mixture of a polymer solution and a detergent to form an emulsion, wherein the polymer solution comprises a polymer selected from the group consisting of a poly(α -hydroxy acid), a polyhydroxy butyric acid, a polycaprolactone, a polyorthoester, a polyanhydride, and a polycyanoacrylate, wherein the polymer is present at a concentration of about 1% to about 30% in an organic solvent, and wherein the detergent is present in the mixture at a weight to weight detergent to

organic solvent from the emulsion, to form said microparticle; and (c) adsorbing the vector construct to the surface of the microparticle, wherein said vector construct selected from the group consisting of an ELVIS vector and an RNA vector construct.

52. The method of claim 51, wherein the vector construct is an ELVIS vector or an RNA vector construct, and comprises a heterologous nucleic acid sequence encoding a member selected from the group consisting of a pharmaceutical, a polypeptide, a hormone, an enzyme, a transcription or translation mediator, an intermediate in a metabolic pathway, an immunomodulator, an antigen, and an adjuvant.

53. The method of claim 52, wherein the heterologous nucleic acid sequence encodes an antigen selected from the group consisting of HIV gp120, HIV gp140, HIV p24gag, HIV p55gag, and Influenza A hemagglutinin antigen.

54. The method of claim 53, wherein the antigen is HIV gp140.

55. A microparticle made according to the method of claim 51.

56. A microparticle composition comprising the microparticle of claim 55 and a pharmaceutically acceptable excipient.

57. A method of inducing an immune response in a host animal comprising administering to said animal the microparticle composition of claim 47.

58. The method of claim 57 wherein said mammal is a human.

59. A method of immunizing a host animal against a viral, bacterial, or parasitic infection comprising administering to said animal the microparticle composition of any of claim 47.

60. The method of claim 59 wherein said mammal is a human.

61. A method of inducing a Th1 immune response in a host animal comprising administering to said animal the microparticle composition of claim 47.

62. The method of claim 61 wherein said mammal is a human.

63. A method of inducing a CTL immune response in a host animal comprising administering to said animal the microparticle composition of claim 47.

64. The method of claim 63 wherein said mammal is a human.

65. A method of delivering a therapeutically effective amount of a macromolecule to a host animal comprising the step of administering to the vertebrate subject a microparticle composition of claim 47.

66. The method of claim 65 wherein said mammal is a human.

67. A method of treating a host animal having a viral, bacterial, or parasitic infection comprising administering to said animal the microparticle composition of claim 47 in an amount effective to reduce the level of infection thereof.

68. The method of claim 67 wherein said mammal is a human.

69. Use of a microparticle composition of claim 47 for treatment of a disease.

70. Use of a microparticle composition of claim 47 for a vaccine.

71. Use of a microparticle composition of claim 47 for raising an immune response.

72. The microparticle of claim 39, wherein said heterologous nucleic acid sequence encodes an HIV gag polypeptide and comprises a sequence having at least 90% identity to a sequence selected from the group consisting of nucleotides 844-903 of SEQ ID NOs:63, nucleotides 841-900 of SEQ ID NO:64, nucleotides 1513-2547 of SEQ ID NO:65, nucleotides 1210-1353 of SEQ ID NO:66, nucleotides 1213-1353 of SEQ ID NO:67, and nucleotides 82-1512 of SEQ ID NO:68.

73. The microparticle of claim 39, wherein said heterologous nucleic acid sequence encodes an HIV envelope polypeptide and comprises a sequence having at least 90% identity to a sequence selected from the group consisting of nucleotides 844-903 of SEQ ID NOs:63, nucleotides 841-900 of SEQ ID NO:64, nucleotides 1513-2547 of SEQ ID NO:65, nucleotides 1210-1353 of SEQ ID NO:66, nucleotides 1213-1353 of SEQ ID

74. The method of claim 53, wherein said heterologous nucleic acid sequence encodes an HIV gag polypeptide and comprises a sequence having at least 90% identity to a sequence selected from the group consisting of nucleotides 844-903 of SEQ ID NOs:63, nucleotides 841-900 of SEQ ID NO:64, nucleotides 1513-2547 of SEQ ID NO:65, nucleotides 1210-1353 of SEQ ID NO:66, nucleotides 1213-1353 of SEQ ID NO:67, and nucleotides 82-1512 of SEQ ID NO:68.

75. The method of claim 53, wherein said heterologous nucleic acid sequence encodes an HIV envelope polypeptide and comprises a sequence having at least 90% identity to a sequence selected from the group consisting of nucleotides 844-903 of SEQ ID NOs:63, nucleotides 841-900 of SEQ ID NO:64, nucleotides 1513-2547 of SEQ ID NO:65, nucleotides 1210-1353 of SEQ ID NO:66, nucleotides 1213-1353 of SEQ ID NO:67, and nucleotides 82-1512 of SEQ ID NO:68.

76. The microparticle of claim 27, wherein (a) the oil is a terpenoid and (b) the one or more emulsifying agents comprise one or more non-ionic detergents and one or more cationic detergents.

77. The microparticle of claim 76, wherein the oil is squalene and the one or more emulsifying agents comprise: a polyoxyethylene sorbitan fatty acid ester, a sorbitan fatty acid ester, and DOTAP.

L5 ANSWER 50 OF 112 USPTAFULL on STN

2003:180726 Method for rapid detection and identification of bioagents.

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US 2003124556 A1 20030703

APPLICATION: US 2002-156608 A1 20020524 (10)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Method for detecting and identifying unknown bioagents, including bacteria, viruses and the like, by a combination of nucleic acid amplification and molecular weight determination using primers which hybridize to conserved sequence regions of nucleic acids derived from a bioagent and which bracket variable sequence regions that uniquely identify the bioagent. The result is a "base composition signature" (BCS) which is then matched against a database of base composition signatures; by which the bioagent is identified.

CLM What is claimed is:

1. A method of identifying an unknown bioagent comprising: (a) contacting nucleic acid from said bioagent with at least one pair of **oligonucleotide primers** which hybridize to sequences of said nucleic acid, wherein said sequences flank a variable nucleic acid sequence of the bioagent; (b) amplifying said variable nucleic acid sequence to produce an amplification product; (c) determining the molecular mass of said amplification product; and (d) comparing said molecular mass to one or more molecular masses of amplification products obtained by performing steps (a)-(c) on a plurality of known organisms, wherein a match identifies said unknown bioagent.

2. The method of claim 1, wherein said sequences to which said at least one pair of **oligonucleotide primers** hybridize are highly conserved.

3. The method of claim 1, wherein said amplifying step comprises **polymerase chain reaction**.

4. The method of claim 1, wherein said amplifying step comprises ligase chain reaction or strand displacement amplification.

5. The method of claim 1, wherein said bioagent is a bacterium, virus, cell or spore.

6. The method of claim 1, wherein said nucleic acid is ribosomal RNA.

7. The method of claim 1, wherein said nucleic acid encodes RNase P or an RNA-dependent RNA polymerase.

8. The method of claim 1, wherein said amplification product is ionized prior to molecular mass determination.

9. The method of claim 1, further comprising the step of isolating nucleic acid from said bioagent prior to contacting said nucleic acid with said at least one pair of **oligonucleotide primers**.

10. The method of claim 1, further comprising the step of performing

comparing the results to one or more molecular mass amplification product obtained by performing steps (a)-(c) on a different plurality of known organisms from those in step (d).

11. The method of claim 1, wherein said one or more molecular masses are contained in a database of molecular masses.

12. The method of claim 1, wherein said amplification product is ionized by electrospray ionization, matrix-assisted laser desorption or fast atom bombardment.

13. The method of claim 1, wherein said molecular mass is determined by mass spectrometry.

14. The method of claim 11, wherein said mass spectrometry is selected from the group consisting of Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS), ion trap, quadrupole, magnetic sector, time of flight (TOF), Q-TOF and triple quadrupole.

15. The method of claim 1, further comprising performing step (b) in the presence of an analog of adenine, thymidine, guanosine or cytidine having a different molecular weight than adenosine, thymidine, guanosine or cytidine.

16. The method of claim 1, wherein said **oligonucleotide primer** comprises a base analog at positions 1 and 2 of each triplet within said **primer**, wherein said base analog binds with increased affinity to its complement compared to the native base.

17. The method of claim 16, wherein said **primer** comprises a universal base at position 3 of each triplet within said **primer**.

18. The method of claim 16, wherein said base analog is selected from the group consisting of 2,6-diaminopurine, propyne T, propyne G, phenoxazines and G-clamp.

19. The method of claim 16, wherein said universal base is selected from the group consisting of inosine, guanidine uridine, 5-nitroindole, 3-nitropyrrole, dP, dK, and 1-(2-deoxy- β -D-ribofuranosyl)-imidazole-4-carboxamide.

20. A method of identifying an unknown bioagent comprising: contacting nucleic acid from said bioagent with at least one pair of **oligonucleotide primers** which hybridize to sequences of said nucleic acid, wherein said sequences flank a variable nucleic acid sequence; amplifying said variable nucleic acid sequence to produce an amplification product; determining the base composition of said amplification product; and comparing said base composition to one or more base compositions of amplification products obtained by performing steps (a)-(c) on a plurality of known organisms, wherein a match identifies said unknown bioagent.

21. The method of claim 20, wherein said sequences to which said at least one pair of **oligonucleotide primers** hybridize are highly conserved.

22. The method of claim 20, wherein said amplifying step comprises **polymerase chain reaction**.

23. The method of claim 20, wherein said amplifying step comprises ligase chain reaction or strand displacement amplification.

24. The method of claim 20, wherein said bioagent is a bacterium, virus, cell or spore.

25. The method of claim 20, wherein said nucleic acid is ribosomal RNA.

26. The method of claim 20, wherein said nucleic acid encodes RNase P or an RNA-dependent RNA polymerase.

27. The method of claim 20, wherein said amplification product is ionized prior to base composition determination.

28. The method of claim 20, further comprising the step of isolating nucleic acid from said bioagent prior to contacting said nucleic acid with said at least one pair of **oligonucleotide primers**.

29. The method of claim 20, further comprising the step of performing steps (a)-(d) using a different **oligonucleotide primer** pair and comparing the results to one or more base compositions of amplification product obtained by performing steps (a)-(c) on a different plurality of known organisms from those in step (d).

30. The method of claim 20, wherein said one or more base composition signatures are contained in a database of base composition signatures.
31. The method of claim 20, wherein said amplification product is ionized by electrospray ionization, matrix-assisted laser desorption or fast atom bombardment.
32. The method of claim 20, wherein said base composition signature is determined by mass spectrometry.
33. The method of claim 32, wherein said mass spectrometry is selected from the group consisting of Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS), ion trap, quadrupole, magnetic sector, time of flight (TOF), q-TOF and triple quadrupole.
34. The method of claim 20, further comprising performing step (b) in the presence of an analog of adenine, thymidine, guanosine or cytidine having a different molecular weight than adenosine, thymidine, guanosine or cytidine.
35. The method of claim 20, wherein said **oligonucleotide primer** comprises a base analog at positions 1 and 2 of each triplet within said **primer**, wherein said base analog binds with increased affinity to its complement compared to the native base.
36. The method of claim 35, wherein said **primer** comprises a universal base at position 3 of each triplet within said **primer**.
37. The method of claim 35, wherein said base analog is selected from the group consisting of 2,6-diaminopurine, propyne T, propyne G, phenoxazines and G-clamp.
38. The method of claim 36, wherein said universal base is selected from the group consisting of inosine, guanidine uridine, 5-nitroindole, 3-nitropyrrole, dP, dK, and 1-(2-deoxy- β -D-ribofuranosyl)-imidazole-4-carboxamide.
39. A method for detecting a single nucleotide polymorphism in an individual, comprising the steps of: isolating nucleic acid from said individual; contacting said nucleic acid with **oligonucleotide primers** which hybridize to regions of said nucleic acid which flank a region comprising said potential polymorphism; amplifying said region to produce an amplification product; determining the molecular mass of said amplification product; comparing said molecular mass to the molecular mass of said region in an individual known to have said polymorphism, wherein if said molecular masses are the same then said individual has said polymorphism.
40. The method of claim 39, wherein said polymorphism is associated with a disease.
41. The method of claim 39, wherein said polymorphism is a blood group antigen.
42. The method of claim 39, wherein said amplification step is the **polymerase chain reaction**.
43. The method of claim 39, wherein said amplification step is ligase chain reaction or strand displacement amplification.
44. The method of claim 39, wherein said amplification product is ionized prior to mass determination.
45. The method of claim 39, wherein said amplification product is ionized by electrospray ionization, matrix-assisted laser desorption or fast atom bombardment.
46. The method of claim 39, wherein said **primers** hybridize to conserved sequences.
47. The method of claim 39, wherein said molecular mass is determined by mass spectrometry.
48. The method of claim 47, wherein said mass spectrometry is selected from the group consisting of Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS), ion trap, quadrupole, magnetic sector, time of flight (TOF), Q-TOF and triple quadrupole.

2003:173916 Induction of viral mutation by incorporation of miscoding

ribonucleoside analogs into viral RNA.

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US 2003119764 A1 20030626

APPLICATION: US 2000-522373 A1 20000310 (9)

PRIORITY: US 1996-29404P 19961028 (60)

US 1997-40535P 19970227 (60)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention is directed to the identification and use of ribonucleoside analogs to induce the mutation of an RNA virus, including BVDV, HIV and HCV, or a virus which otherwise replicates through an RNA intermediate. The increase in the mutation rate of the virus results in reduced viability of progeny generations of the virus, thereby inhibiting viral replication. In addition to these methods and related compositions, the invention provides methods and combinatorial chemistry libraries for screening ribonucleoside analogs for mutagenic potential.

CLM What is claimed is:

1. A method of increasing the mutation rate of a virus, comprising administering an RNA nucleoside analog to a virally infected cell, wherein the analog is incorporated by a polymerase into an RNA copy of a genomic nucleic acid encoding the virus, said analog replacing a first natural occurring nucleotide having a first complementary nucleotide wherein said analog complements a second nucleotide which is other than the first nucleotide, thereby inducing the virus to mutate.

2. The method of claim 1, wherein the RNA nucleoside analog replaces uracil.

3. The method of claim 1, wherein the RNA nucleoside analog replaces adenine.

4. The method of claim 1, wherein the RNA nucleoside analog replaces cytidine.

5. The method of claim 1, wherein the RNA nucleoside analog replaces guanine.

6. The method of claim 1, wherein the RNA nucleoside analog is incorporated by the polymerase into the RNA copy of the genomic nucleic acid with an efficiency at least about 0.1% that of a naturally occurring complementary nucleic acid.

7. The method of claim 1, wherein the method further includes the proviso that the RNA nucleoside analog is not ribavirin or a 5-halo analog of 1-a-D-ribofuranosylimidazole-4-carboxamide.

8. The method of claim 1, wherein the RNA analog is a non-chain terminating analog.

9. The method of claim 1, wherein the method further includes the proviso that if the virus is HIV, then the RNA nucleoside analog is not HEPT or a 2',5'-bis-O-sialylated-3'-spiro-substituted (TSAO) adenine, hypoxanthine, N1-alkyl-hypoxanthine, or xanthine or a nucleoside analog that is incorporated and extended at high efficiency by reverse transcriptase of HIV.

10. The method of claim 1, wherein the nucleoside analog is selected from the group consisting of N4-aminocytidine, N1-methyl-N4-aminocytidine, 3,N4-ethenocytidine, 3-methylcytidine, 5-hydroxycytidine, N4-dimethylcytidine, 5-(2-hydroxyethyl)cytidine, 5-chlorocytidine, 5-bromocytidine, N4-methyl-N4-aminocytidine, 5-aminocytidine, 5-nitrosocytidine, 5-(hydroxyalkyl)-cytidine, 5-(thioalkyl)-cytidine and cytidine glycol, 5-hydroxyuridine, 3-hydroxyethyluridine, 3-methyluridine, O2-methyluridine, O2-ethyluridine, 5-aminouridine, O4-methyluridine, O4-ethyluridine, O4-isobutyluridine, O4-alkyluridine, 5-nitrosouridine, 5-(hydroxyalkyl)-uridine, and 5-(thioalkyl)-uridine, 1,N6-ethenoadenosine, 3-methyladenosine, and N6-methyladenosine, 8-hydroxyguanosine, O6-methylguanosine, O6-ethylguanosine, O6-isopropylguanosine, 3,N2-ethenoguanosine, O6-alkylguanosine, 8-oxo-guanosine, 2,N3-ethenoguanosine, and 8-aminoguanosine.

11. The method of claim 1, wherein the virus is a retrovirus or a flavivirus.

12. The method of claim 11, wherein the virus is a pestivirus.

13. The method of claim 1, wherein the polymerase is a human polymerase II.

14. The method of claim 1, wherein the cell is in cell culture.
15. The method of claim 1, wherein the cell is in an animal.
16. The method of claim 1, wherein increasing the mutation rate of the virus produces a progressive loss of viability of the virus.
17. The method of claim 1, comprising administration of more than one species of RNA nucleoside analog to the virally infected cell.
18. The method of claim 1, wherein the virus is an RNA virus selected from the group consisting of hepatitis C, coronavirus, influenza, respiratory syncytial virus, BVDV, and dengue fever.
19. A viral particle comprising viral genomic RNA, wherein the viral genomic RNA comprises an RNA nucleoside analog.
20. The viral particle of claim 19, wherein the particle is an HIV particle, and HCV particle, or a BVDV particle.
21. The viral particle of claim 19, wherein the nucleoside analog is selected from the group consisting of N⁴-aminocytidine, N¹-methyl-N⁴-aminocytidine, 3,N⁴-ethenocytidine, 3-methylcytidine, 5-hydroxycytidine, N⁴-dimethylcytidine, 5-(2-hydroxyethyl)cytidine, 5-chlorocytidine, 5-bromocytidine, N⁴-methyl-N⁴-aminocytidine, 5-aminocytidine, 5-nitrosocytidine, 5-(hydroxyalkyl)-cytidine, 5-(thioalkyl)-cytidine and cytidine glycol, 5-hydroxyuridine, 3-hydroxyethyluridine, 3-methyluridine, O²-methyluridine, O²-ethyluridine, 5-aminouridine, O⁴-methyluridine, O⁴-ethyluridine, O⁴-isobutyluridine, O⁴-alkyluridine, 5-nitrosouridine, 5-(hydroxyalkyl)-uridine, and 5-(thioalkyl)-uridine, 1,N⁶-ethenoadenosine, 3-methyladenosine, and N⁶-methyladenosine, 8-hydroxyguanosine, O⁶-methylguanosine, O⁶-ethylguanosine, O⁶-isopropylguanosine, 3,N²-ethenoguanosine, O⁶-alkylguanosine, 8-oxo-guanosine, 2,N³-ethenoguanosine, and 8-aminoguanosine.
22. A population of cells comprising a highly variable population of replicated homologous viral nucleic acids.
23. The population of cells of claim 22, wherein the cells are in cell culture.
24. The population of cells of claim 22, wherein the viral nucleic acids are derived from a retrovirus or a flavivirus.
25. A cell comprising a viral genomic nucleic acid, an RNA analog, a cellular mRNA analog and a viral genomic RNA analog.
26. The cell of claim 25, wherein the viral genomic nucleic acid is integrated into the cellular genome.
27. The cell of claim 25, wherein the viral genomic nucleic acid is a retroviral or a flaviviral nucleic acid.
28. The cell of claim 25, wherein the viral genomic nucleic acid is an HIV nucleic acid, a pestivirus nucleic acid, or an HCV virus nucleic acid.
29. The cell of claim 25, wherein the viral genomic nucleic acid is selected from the group consisting of a HIV-1, HIV-2, HTLV-1, HTLV-2, SIV, hepatitis A, hepatitis B, hepatitis C, BVDV, and dengue fever virus.
30. A method of detecting the mutagenic potential of a ribonucleoside analog comprising integrating the ribonucleoside analog into a viral RNA synthesized by a polymerase, and determining whether the incorporation causes a mutation in a progeny virus.
31. The method of claim 30, wherein the ribonucleoside analog is selected from the group consisting of N⁴-aminocytidine, N¹-methyl-N⁴-aminocytidine, 3,N⁴-ethenocytidine, 3-methylcytidine, 5-hydroxycytidine, N⁴-dimethylcytidine, 5-(2-hydroxyethyl)cytidine, 5-chlorocytidine, 5-bromocytidine, N⁴-methyl-N⁴-aminocytidine, 5-aminocytidine, 5-nitrosocytidine, 5-(hydroxyalkyl)-cytidine, 5-(thioalkyl)-cytidine and cytidine glycol, 5-hydroxyuridine, 3-hydroxyethyluridine, 3-methyluridine, O²-methyluridine, O²-ethyluridine, 5-aminouridine, O⁴-methyluridine, O⁴-ethyluridine, O⁴-isobutyluridine, O⁴-alkyluridine, 5-nitrosouridine, 5-(hydroxyalkyl)-uridine, and 5-(thioalkyl)-uridine,

methyladenosine, 8-hydroxyguanosine, O6-methylguanosine, O6-ethylguanosine, O6-isopropylguanosine, 3,N2-ethenoguanosine, O6-alkylguanosine, 8-oxo-guanosine, 2,N3-ethenoguanosine, and 8-aminoguanosine.

32. The method of claim 30, wherein the virus is an RNA virus.

33. The method of claim 30, wherein the virus is a retrovirus or a flavivirus.

34. The method of claim 30, wherein the virus is selected from the group consisting of HIV-1, HIV-2, HTLV-1, HTLV-2, SIV, hepatitis A, hepatitis B, hepatitis C, BVDV, and dengue fever virus.

35. A method of screening for a ribonucleoside analog which is incorporated by a cellular polymerase, comprising incubating the cellular polymerase with the ribonucleoside analog in the presence of a nucleic acid template and detecting whether the ribonucleoside analog is polymerized.

36. The method of claim 35, wherein the cellular polymerase is present in a cell.

37. The method of claim 35, wherein the method further comprises incubating the cellular polymerase with a naturally occurring ribonucleoside.

38. The method of claim 35, wherein the method further comprises comparing the rate of incorporation of the ribonucleoside analog and the naturally occurring ribonucleoside into an RNA polymer.

39. The method of claim 35, wherein the virus is a retrovirus or a flavivirus.

40. The method of claim 35, wherein the cellular polymerase is a mammalian pol II polymerase.

41. A pharmaceutical composition comprising a therapeutically effective dose of an RNA nucleoside analog, wherein the analog is one that in a infected cell with a virus of interest is incorporated by a polymerase into an RNA copy of a genomic nucleic acid encoding the virus, said analog replacing a first natural occurring nucleotide having a first complementary nucleotide wherein said analog complements a second nucleotide which is other than the first nucleotide together with a pharmaceutically acceptable carrier.

42. The pharmaceutical composition of claim 41, wherein the nucleoside analog is selected from the group consisting of N4-aminocytidine, N1-methyl-N4-aminocytidine, 3,N4-ethenocytidine, 3-methylcytidine, 5-hydroxycytidine, N4-dimethylcytidine, 5-(2-hydroxyethyl)cytidine, 5-chlorocytidine, 5-bromocytidine, N4-methyl-N4-aminocytidine, 5-aminocytidine, 5-nitrosocytidine, 5-(hydroxyalkyl)-cytidine, 5-(thioalkyl)-cytidine and cytidine glycol, 5-hydroxyuridine, 3-hydroxyethyluridine, 3-methyluridine, O2-methyluridine, O2-ethyluridine, 5-aminouridine, O4-methyluridine, O4-ethyluridine, O4-isobutyluridine, O4-alkyluridine, 5-nitrosouridine, 5-(hydroxyalkyl)-uridine, and 5-(thioalkyl)-uridine, 1,N6-ethenoadenosine, 3-methyladenosine, and N6-methyladenosine, 8-hydroxyguanosine, O6-methylguanosine, O6-ethylguanosine, O6-isopropylguanosine, 3,N2-ethenoguanosine, O6-alkylguanosine, 8-oxo-guanosine, 2,N3-ethenoguanosine, and 8-aminoguanosine.

43. The pharmaceutical composition of claim 41, wherein the pharmaceutical composition is suitable for oral administration.

44. The pharmaceutical composition of claim 41, wherein the pharmaceutical composition is suitable for parenteral administration.

45. A method of increasing the mutation rate of a virus in an animal comprising administering to the animal a therapeutically effective dose of a mutagenic ribonucleoside analog composition of claim 41.

46. The method of claim 45, wherein the nucleoside analog is selected from the group consisting of N4-aminocytidine, N1-methyl-N4-aminocytidine, 3,N4-ethenocytidine, 3-methylcytidine, 5-hydroxycytidine, N4-dimethylcytidine, 5-(2-hydroxyethyl)cytidine, 5-chlorocytidine, 5-bromocytidine, N4-methyl-N4-aminocytidine, 5-aminocytidine, 5-nitrosocytidine, 5-(hydroxyalkyl)-cytidine, 5-(thioalkyl)-cytidine and cytidine glycol, 5-hydroxyuridine, 3-hydroxyethyluridine, 3-methyluridine, O2-methyluridine,

O4-ethyluridine, O4-isobutyluridine, O4-alkyluridine, 5-nitrosouridine, 5-(hydroxyalkyl)-uridine, and 5-(thioalkyl)-uridine, 1,N6-ethenoadenosine, 3-methyladenosine, and N6-methyladenosine, 8-hydroxyguanosine, O6-methylguanosine, O6-ethylguanosine, O6-isopropylguanosine, 3,N2-ethenoguanosine, O6-alkylguanosine, 8-oxo-guanosine, 2,N3-ethenoguanosine, and 8-aminoguanosine.

47. The method of claim 45, wherein the RNA nucleoside analog is incorporated by a polymerase present in virally infected cells of the animal into an RNA copy of a genomic nucleic acid of the virus with an efficiency at least about 0.1% that of a naturally occurring complementary nucleic acid.

48. The method of claim 45, wherein the animal is a human patient infected with a virus selected from the group consisting of HIV-1, HIV-2, HTLV-1, HTLV-2, hepatitis A, hepatitis B, hepatitis C, and dengue fever virus.

49. The method of claim 45, wherein the animal is a human patient having a disease selected from the group consisting of AIDS, hepatitis B, hepatitis C, T-cell leukemia.

50. The method of claim 45, the animal having a disease selected from the group consisting of feline leukemia virus, feline immunodeficiency virus, BVDV, or vesicular stomatitis virus.

51. A method of identifying a mutagenic ribonucleoside analog, comprising: providing a plurality of ribonucleoside analogs; incorporating a portion of the ribonucleoside analogs into a ribonucleoside polymer using a RNA polymerase; isolating the ribonucleoside polymer; and, determining the chemical composition of a ribonucleoside analogs which is incorporated into the ribonucleoside polymer.

52. The method of claim 51, wherein the method further comprises hydrolyzing the ribonucleoside polymer.

53. The method of claim 51, wherein the polymer is isolated using electrophoresis.

54. The method of claim 51, wherein the method further comprises hydrolyzing the ribonucleoside polymer to yield ribonucleoside analog monomers and determining the structure of the ribonucleoside analog monomers using a technique selected from the group consisting of mass spectroscopy and NMR.

55. A method of making a mutagenic ribonucleoside analog comprising: chemically modifying a nucleotide analog selected from the group consisting of uridine, cytidine, adenosine, guanosine, N4-aminocytidine, N1-methyl-N4-aminocytidine, 3,N4-ethenocytidine, 3-methylcytidine, 5-hydroxycytidine, N4-dimethylcytidine, 5-(2-hydroxyethyl)cytidine, 5-chlorocytidine, 5-bromocytidine, N4-methyl-N4-aminocytidine, 5-aminocytidine, 5-nitrosocytidine, 5-(hydroxyalkyl)-cytidine, 5-(thioalkyl)-cytidine and cytidine glycol, 5-hydroxyuridine, 3-hydroxyethyluridine, 3-methyluridine, O2-methyluridine, O2-ethyluridine, 5-aminouridine, O4-methyluridine, O4-ethyluridine, O4-isobutyluridine, O4-alkyluridine, 5-nitrosouridine, 5-(hydroxyalkyl)-uridine, and 5-(thioalkyl)-uridine, 1,N6-ethenoadenosine, 3-methyladenosine, and N6-methyladenosine, 8-hydroxyguanosine, O6-methylguanosine, O6-ethylguanosine, O6-isopropylguanosine, 3,N2-ethenoguanosine, O6-alkylguanosine, 8-oxo-guanosine, 2,N3-ethenoguanosine, and 8-aminoguanosine to yield a chemically modified ribonucleoside analog; determining whether the chemically modified analog is incorporated by an RNA polymerase into a polyribonucleotide molecule; and, measuring the mutagenic potential of the chemically modified analog.

56. The method of claim 55, wherein the step of chemically modifying the **oligonucleotide** comprises exposing the selected nucleotide analog to oxygen free radicals.

57. A library of nucleoside analogs made by the method of claim 55, wherein each nucleoside analog comprises a random chemical substituent linked to a group selected from the group consisting of uridine, cytidine, guanosine, adenosine, N4-aminocytidine, N1-methyl-N4-aminocytidine, 3,N4-ethenocytidine, 3-methylcytidine, 5-hydroxycytidine, N4-dimethylcytidine, 5-(2-hydroxyethyl)cytidine, 5-chlorocytidine, 5-bromocytidine, N4-methyl-N4-aminocytidine, 5-aminocytidine,

cytidine glycol, 5-hydroxyuridine, 3-hydroxyethyluridine, 3-methyluridine, O2-methyluridine, O2-ethyluridine, 5-aminouridine, O4-methyluridine, O4-ethyluridine, O4-isobutyluridine, O4-alkyluridine, 5-nitrosouridine, 5-(hydroxyalkyl)-uridine, and 5-(thioalkyl)-uridine, 1,N6-ethenoadenosine, 3-methyladenosine, and N6-methyladenosine, 8-hydroxyguanosine, O6-methylguanosine, O6-ethylguanosine, O6-isopropylguanosine, 3,N2-ethenoguanosine, O6-alkylguanosine, 8-oxo-guanosine, 2,N3-ethenoguanosine, and 8-aminoguanosine.

58. The library of claim 57, wherein a plurality of the nucleoside analogs are polymerized.

59. The library of claim 57, wherein the library further comprises an RNA polymerase.

60. The library of claim 57, wherein the library comprises between about 5 and 1,000,000 different analogs.

61. The library of claim 57, wherein the cellular RNA polymerase is human RNA polymerase II.

62. A kit comprising a container and one or more of the following components: a control mutagenic RNA analog, a test mutagenic RNA analog, an RNA polymerase, reagents for detecting incorporation of the RNA analog by the RNA polymerase, and instructions in the use of the kit components for detecting the mutagenic potential of the test mutagenic analog as compared to the control mutagenic RNA analog.

63. A method of increasing the mutation rate of a virus in an animal comprising administering to the animal (a) a therapeutically effective dose of a ribonucleoside analog to a virally infected cell, wherein the analog is incorporated by a polymerase into an RNA copy of a genomic nucleic acid encoding the virus, said analog replacing a first natural occurring nucleotide having a first complementary nucleotide wherein said analog complements a second nucleotide which is other than the first nucleotide, in combination with (b) a drug that reduces the concentration of the first natural occurring nucleotide.

64. A method of increasing the mutation rate of a virus, comprising administering a free base selected from the group comprising adenine, cytosine, guanine, uracil and thymine to a virally infected cell, wherein the base is incorporated by a polymerase into an RNA or DNA copy of a genomic nucleic acid encoding the virus, said base replacing a first natural occurring nucleotide having a first complementary nucleotide wherein said base complements a second nucleotide which is other than the first nucleotide, thereby inducing the virus to mutate.

65. The ribonucleoside analog identified by the method of claim 51.

66. The method of claim 1, wherein the RNA nucleoside analog is an enantio-specific nucleoside analog.

67. The pharmaceutical composition of claim 41, wherein the RNA nucleoside analog is an enantio-specific nucleoside analog.

L5 ANSWER 52 OF 112 USPTAFULL on STN

2003:152910 DNA CONSTRUCT, COMPOSITION, FORMULATIONS & METHODS FOR MAKING THE CONSTRUCT & FOR MODULATING EXPRESSION.

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US 2003104576 A1 20030605

APPLICATION: US 1994-319974 A1 19941007 (8)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Disclosed are attenuated viruses, not naturally occurring, that contain one or more additional methylation sites in the genome of the virus compared to the corresponding wild-type virus. Preferably, the methylation sites are added into the genome of the virus by introducing an additional CG segment into the genome by means of a silent mutation. The attenuated viruses are useful for producing an immune response, including both the production of antibodies in animals for diagnostic use and the induction of protective immunity in a subject. Pharmaceutical formulations and methods of making the attenuated viruses are also disclosed.

CLM What is claimed is:

1. An attenuated virus, not naturally occurring, containing at least 1 additional methylation site in the genome of said virus compared to the corresponding wild-type virus.

2. An attenuated virus according to claim 1, said virus comprising a

3. An attenuated virus of claim 1, containing at least 10 additional methylation sites over the corresponding wild-type virus.
4. An attenuated virus of claim 1, containing at least 100 additional methylation sites over the corresponding wild-type virus.
5. An attenuated virus of claim 1 wherein said methylation site is a CG segment.
6. An attenuated virus according to claim 1, wherein said virus is a DNA virus.
7. An attenuated virus according to claim 1, wherein said virus is a retrovirus.
8. An attenuated virus of claim 1 wherein said virus is a retrovirus selected from the group consisting of B-type retroviruses, C-type retroviruses, D-type retroviruses, Lentiviruses, T-cell leukemia viruses, and foamy viruses.
9. An attenuated virus of claim 1, wherein said virus is HIV-1.
10. An attenuated virus of claim 1, wherein said virus is SIV.
11. An attenuated virus of claim 1, wherein said virus is HTLV-1.
12. An attenuated virus of claim 1, wherein said virus is a retrovirus and wherein an attenuating deletion mutation is included therein.
13. A DNA encoding a virus of claim 1.
14. An expression vector containing a DNA of claim 13.
15. An expression vector of claim 14, wherein said expression vector is a Baculovirus.
16. A host cell containing a DNA of claim 13 and capable of expressing the encoded virus, which host cell does not methylate said DNA sufficient to inactivate the expression of the encoded viral genome.
17. A host cell according to claim 16, which host cell lacks capacity to methylate DNA because of treatment of said host cell with a methylation inhibitor.
18. A host cell according to claim 17 wherein said methylation inhibitor is 5-azadeoxycytidine or 5-azacytidine.
19. A pharmaceutical formulation comprising a virus according to claim 1 in combination with a pharmaceutically acceptable carrier.
20. A formulation according to claim 19, wherein said formulation is an oral formulation.
21. A formulation according to claim 19, wherein said formulation is a parenterally injectable vaccine formulation.
22. A formulation according to claim 19, wherein said formulation is an inhalation formulation.
23. A method of producing an immune response in a subject, comprised of administering a virus of claim 1 to said subject in an amount effective to produce an immune response in said subject.
24. A method according to claim 23, wherein said administering step is carried out by orally administering said virus to said subject.
25. A method according to claim 23, wherein said administering step is carried out by parenterally injecting said virus into said subject.
26. A method according to claim 23, wherein said subject is an animal subject.
27. A method according to claim 23, wherein said subject is a human subject.
28. A method of making an attenuated virus, not naturally occurring, containing at least 1 additional methylation site in the genome of said virus compared to the corresponding wild-type virus; said method comprising: providing a host cell containing an expression vector, said expression vector containing a DNA encoding said attenuated virus, which host cell does not methylate said DNA sufficient to inactivate the

virus in said host cell.

29. A method according to claim 28, the genome of said virus containing at least 10 additional methylation sites over the corresponding wild-type virus.

30. A method according to claim 28, wherein said virus is a DNA virus.

31. A method according to claim 28, wherein said virus is a retrovirus.

32. A method according to claim 28, wherein said expression vector is a Baculovirus.

33. A method according to claim 28, wherein said host cell is an insect cell.

34. A method according to claim 28, wherein said host cell is a mammalian cell.

35. An **oligonucleotide** probe useful for distinguishing between (i) an attenuated virus, not naturally occurring, containing at least 1 additional methylation site in the genome of said virus compared to the corresponding wild-type virus, and (ii) said corresponding wild-type virus, said **oligonucleotide** probe selected from the group consisting of: (a) **oligonucleotide** probes that selectively hybridize to the nucleic acid of an attenuated virus of (i) above, and which do not hybridize to the nucleic acid of the wild-type virus of (ii) above under the same hybridization conditions; and (b) **oligonucleotide** probes that selectively hybridize to the nucleic acid of a wild-type virus of (ii) above, and which do not hybridize to the nucleic acid of the attenuated virus of (i) above under the same hybridization conditions.

36. An **oligonucleotide** probe according to claim 35 conjugated to a detectable group.

37. An **oligonucleotide** probe according to claim 35, wherein said probe is a **PCR** extension **primer**.

L5 ANSWER 53 OF 112 USPATFULL on STN

2003:145924 Packaging of immunostimulatory substances into virus-like particles: method of preparation and use.

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US 2003099668 A1 20030529

APPLICATION: US 2002-244065 A1 20020916 (10)

PRIORITY: US 2001-318994P 20010914 (60)

US 2002-374145P 20020422 (60)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention relates to the finding that virus like particles (VLPs) can be loaded with immunostimulatory substances, in particular with DNA oligonucleotides containing non-methylated C and G (CpGs). Such CpG-VLPs are dramatically more immunogenic than their CpG-free counterparts and induce enhanced B and T cell responses. The immune response against antigens optionally coupled, fused or attached otherwise to the VLPs is similarly enhanced as the immune response against the VLP itself. In addition, the T cell responses against both the VLPs and antigens are especially directed to the Th1 type. Antigens attached to CpG-loaded VLPs may therefore be ideal vaccines for prophylactic or therapeutic vaccination against allergies, tumors and other self-molecules and chronic viral diseases.

CLM What is claimed is:

1. A composition for enhancing an immune response in an animal comprising: (a) a virus-like particle; and (b) an immunostimulatory substance; wherein said immunostimulatory substance is bound to said virus-like particle.

2. The composition of claim 1 further comprising at least one antigen, wherein said antigen is bound to said virus-like particle.

3. The composition of claim 1, wherein said immunostimulatory substance is a toll-like receptor activating substance.

4. The composition of claim 1, wherein said immunostimulatory substance is a cytokine secretion inducing substance.
5. The composition of claim 3, wherein said toll-like receptor activating substance is selected from the group consisting of, or alternatively consists essentially of: (a) immunostimulatory nucleic acids; (b) peptidoglycans; (c) lipopolysaccharides; (d) lipoteichoic acids; (e) imidazoquinoline compounds; (f) flagellines; (g) lipoproteins; (h) immunostimulatory organic molecules; (i) unmethylated CpG-containing **oligonucleotides**; and (j) any mixtures of at least one substance of (a), (b), (c), (d), (e), (f), (g), (h) and/or (i).
6. The composition of claim 5, wherein said immunostimulatory nucleic acid is selected from the group consisting of, or alternatively consists essentially of: (a) ribonucleic acids; (b) deoxyribonucleic acids; (c) chimeric nucleic acids; and (d) any mixtures of at least one nucleic acid of (a), (b) and/or (c).
7. The composition of claim 6, wherein said ribonucleic acid is poly-(I:C) or a derivative thereof.
8. The composition of claim 6, wherein said deoxyribonucleic acid is selected from the group consisting of, or alternatively consists essentially of: (a) unmethylated CpG-containing **oligonucleotides**; and (b) **oligonucleotides** free of unmethylated CpG motifs.
9. The composition of claim 1, wherein said immunostimulatory substance is an unmethylated CpG-containing **oligonucleotide**.
10. The composition of claim 1, wherein said virus-like particle lacks a lipoprotein-containing envelope.
11. The composition of claim 1, wherein said virus-like particle is a recombinant virus-like particle.
12. The composition of claim 11, wherein said virus-like particle is selected from the group consisting of: (a) recombinant proteins of Hepatitis B virus; (b) recombinant proteins of measles virus; (c) recombinant proteins of Sinbis virus; (d) recombinant proteins of Rotavirus; (e) recombinant proteins of Foot-and-Mouth-Disease virus; (f) recombinant proteins of Retrovirus; (g) recombinant proteins of Norwalk virus; (h) recombinant proteins of human Papilloma virus; (i) recombinant proteins of BK virus; (j) recombinant proteins of bacteriophages; (k) recombinant proteins of RNA-phages; (l) recombinant proteins of Q β -phage; (m) recombinant proteins of GA-phage (n) recombinant proteins of fr-phage; (o) recombinant proteins of AP 205-phage; (p) recombinant proteins of Ty; and (q) fragments of any of the recombinant proteins from (a) to (p).
13. The composition of claim 12, wherein said virus-like particle is the Hepatitis B virus core protein.
14. The composition of claim 12, wherein said virus-like particle is the BK virus VP 1 protein.
15. The composition of claim 1, wherein said virus-like particle comprises recombinant proteins, or fragments thereof, of a RNA-phage.
16. The composition of claim 15, wherein said RNA-phage is selected from the group consisting of: (a) bacteriophage Q β ; (b) bacteriophage R17; (c) bacteriophage fr; (d) bacteriophage GA; (e) bacteriophage SP; (f) bacteriophage MS2; (g) bacteriophage M11; (h) bacteriophage MX1; (i) bacteriophage NL95; (k) bacteriophage f2; (l) bacteriophage PP7; and (m) bacteriophage AP205.
17. The composition of claim 1, wherein said virus-like particle comprises recombinant proteins, or fragments thereof, of RNA-phage Q β .
18. The composition of claim 1, wherein said virus-like particle comprises recombinant proteins, or fragments thereof, of RNA-phage AP 205.
19. The composition of claim 9, wherein said unmethylated CpG-containing **oligonucleotide** comprises the sequence: X₁X₂CGX₃X₄ 43' wherein X₁, X₂, X₃, and X₄ are any nucleotide.
20. The composition of claim 19, wherein at least one of said nucleotide X₁, X₂, X₃, and X₄ has a phosphate backbone modification.

21. The composition of claim 9, wherein said unmethylated CpG-containing **oligonucleotide** comprises, or alternatively consists essentially of, or alternatively consists of the sequence selected from the group consisting of: (a) TCCATGACGTTCTGAATAAT; (b) TCCATGACGTTCTGACGTT; (c) GGGGTCAACGTTGAGGGGG; (d) GGGGGGGGGGACGATCGTGGGGGGGGG; and (e) "dsCpG-253" as described in Table 1.

22. The composition of claim 21, wherein said unmethylated CpG-containing **oligonucleotide** contains one or more phosphorothioate modifications of the phosphate backbone or wherein each phosphate moiety of said phosphate backbone of said **oligonucleotide** is a phosphorothioate modification.

23. The composition of claim 9, wherein said unmethylated CpG-containing **oligonucleotide** is palindromic.

24. The composition of claim 23, wherein said palindromic unmethylated CpG-containing **oligonucleotide** comprises, or alternatively consists essentially of, or alternatively consists of the sequence GGGGTCAACGTTGAGGGGG.

25. The composition of claim 24, wherein said palindromic unmethylated CpG-containing **oligonucleotide** contains one or more phosphorothioate modifications of the phosphate backbone or wherein each phosphate moiety of said phosphate backbone of said **oligonucleotide** is a phosphorothioate modification.

26. The composition of claim 1, wherein said immunostimulatory substance is non-covalently bound to said virus-like particle.

27. The composition of claim 9, wherein said unmethylated CpG-containing **oligonucleotide** is non-covalently bound to said virus-like particle.

28. The composition of claim 5, wherein said immunostimulatory nucleic acid is bound to a virus-like particle site selected from the group consisting of an **oligonucleotide** binding site, a DNA binding site and a RNA binding site.

29. The composition of claim 9, wherein said unmethylated CpG-containing **oligonucleotide** is bound to a virus-like particle site selected from the group consisting of an **oligonucleotide** binding site, a DNA binding site and a RNA binding site.

30. The composition of claim 29, wherein said **oligonucleotide** binding site is a non-naturally occurring **oligonucleotide** binding site.

31. The composition of claim 29, wherein said virus-like particle site comprises an arginine-rich repeat.

32. The composition of claim 5, wherein said immunostimulatory nucleic acid contains one or more phosphorothioate modifications of the phosphate backbone or wherein each phosphate moiety of said phosphate backbone of said **oligonucleotide** is a phosphorothioate modification.

33. The composition of claim 9, wherein said unmethylated CpG-containing **oligonucleotide** contains one or more phosphorothioate modifications of the phosphate backbone or wherein each phosphate moiety of said phosphate backbone of said **oligonucleotide** is a phosphorothioate modification.

34. The composition of claim 5, wherein said immunostimulatory nucleic acid, and preferably said unmethylated CpG-containing **oligonucleotide** comprises about 6 to about 100,000 nucleotides.

35. The composition of claim 34, wherein said immunostimulatory nucleic acid, and preferably said unmethylated CpG-containing **oligonucleotide** comprises about 6 to about 2000 nucleotides.

36. The composition of claim 35, wherein said immunostimulatory nucleic acid, and preferably said unmethylated CpG-containing **oligonucleotide** comprises about 20 to about 2000 nucleotides.

37. The composition of claim 36, wherein said immunostimulatory nucleic acid, and preferably said unmethylated CpG-containing **oligonucleotide** comprises about 20 to about 300 nucleotides.

38. The composition of claim 37, wherein said immunostimulatory nucleic acid, and preferably said unmethylated CpG-containing **oligonucleotide** comprises 20 to 100 nucleotides.

39. The composition of claim 5, wherein said immunostimulatory nucleic acid, and preferably said unmethylated CpG-containing **oligonucleotide**

40. The composition of claim 39, wherein said immunostimulatory nucleic acid, and preferably said unmethylated CpG-containing **oligonucleotide** comprises more than 100 to about 1000 nucleotides.

41. The composition of claim 40, wherein said immunostimulatory nucleic acid, and preferably said unmethylated CpG-containing **oligonucleotide** comprises more than 100 to about 500 nucleotides.

42. The composition of claim 5, wherein said immunostimulatory nucleic acid, and preferably said unmethylated CpG-containing **oligonucleotide** is a recombinant **oligonucleotide**.

43. The composition of claim 5, wherein said immunostimulatory nucleic acid, and preferably said unmethylated CpG-containing **oligonucleotide** is a genomic **oligonucleotide**.

44. The composition of claim 5, wherein said immunostimulatory nucleic acid, and preferably said unmethylated CpG-containing **oligonucleotide** is a synthetic **oligonucleotide**.

45. The composition of claim 5, wherein said immunostimulatory nucleic acid, and preferably said unmethylated CpG-containing **oligonucleotide** is a plasmid-derived **oligonucleotide**.

46. The composition of claim 5, wherein said immunostimulatory nucleic acid, and preferably said unmethylated CpG-containing **oligonucleotide** is a single-stranded **oligonucleotide**.

47. The composition of claim 5, wherein said immunostimulatory nucleic acid, and preferably said unmethylated CpG-containing **oligonucleotide** is a double-stranded **oligonucleotide**.

48. The composition of claim 2, wherein said at least one antigen or antigenic determinant is bound to said virus-like particle by at least one covalent bond.

49. The composition of claim 2, wherein said at least one antigen or antigenic determinant is bound to said virus-like particle by at least one covalent bond, and wherein said covalent bond is a non-peptide bond.

50. The composition of claim 2, wherein said at least one antigen or antigenic determinant is fused to said virus-like particle.

51. The composition of claim 2, wherein said antigen or antigenic determinant further comprises at least one second attachment site being selected from the group consisting of: (a) an attachment site not naturally occurring with said antigen or antigenic determinant; and (b) an attachment site naturally occurring with said antigen or antigenic determinant.

52. The composition of claim 2 further comprising an amino acid linker, wherein said amino acid linker comprises, or alternatively consists of, said second attachment site.

53. The composition of claim 2, wherein said antigen is selected from the group consisting of: (a) polypeptides; (b) carbohydrates; (c) steroid hormones; and (d) organic molecules.

54. The composition of claim 53, wherein said antigen is an organic molecule.

55. The composition of claim 54, wherein said organic molecule is selected from the group consisting of: (a) codeine; (b) fentanyl; (c) heroin; (d) morphium; (e) amphetamine; (f) cocaine; (g) methylenedioxymethamphetamine; (h) methamphetamine; (i) methylphenidate; (j) nicotine; (k) LSD; (l) mescaline; (m) psilocybin; and (n) tetrahydrocannabinol.

56. The composition of claim 2, wherein said antigen is a recombinant antigen.

57. The composition of claim 2, wherein said antigen is derived from the group consisting of: (a) viruses; (b) bacteria; (c) parasites; (d) prions; (e) tumors; (f) self-molecules; (g) non-peptidic haptens; (h) allergens; and (i) hormones.

58. The composition of claim 57, wherein said antigen is a tumor antigen.

59. The composition of claim 58, wherein said tumor antigen is selected from the group consisting of: (a) Her2; (b) GD2; (c) EGF-R; (d) CEA;

melanoma protein melan-A/MART-1; (i) tyrosinase; (j) NA 17-A nt protein; (k) MAGE-3 protein; (l) p53 protein; (m) HPV16 E7 protein; and (n) antigenic fragments of any of the tumor antigens from (a) to (m).

60. The composition of claim 2, wherein said antigen is bound to said virus-like particle by way of a linking sequence.

61. The composition of claim 60, wherein said virus-like particle is the Hepatitis B virus core protein.

62. The composition of claim 60, wherein said virus-like particle is the BK virus core protein.

63. The composition of claim 2, wherein said antigen is a cytotoxic T cell epitope, a Th cell epitope or a combination of at least two of said epitopes, wherein said at least two epitopes are bound directly or by way of a linking sequence.

64. The composition of claim 63, wherein said cytotoxic T cell epitope is a viral or a tumor cytotoxic T cell epitope.

65. The composition of claim 63, wherein said antigen is bound to said virus-like particle by way of a linking sequence.

66. The composition of claim 63, wherein said virus-like particle is the Hepatitis B virus core protein.

67. The composition of claim 66, wherein said cytotoxic T cell epitope is fused to the C-terminus of said Hepatitis B virus core protein.

68. The composition of claim 67, wherein said cytotoxic T cell epitope is fused to the C-terminus of said Hepatitis B virus core protein by way of a linking sequence.

69. The composition of claim 63, wherein said virus-like particle is the BK virus VP 1 protein.

70. The composition of claim 69, wherein said cytotoxic T cell epitope is fused to the C-terminus of said BK virus VP1 protein.

71. The composition of claim 70, wherein said cytotoxic T cell epitope is fused to the C-terminus of said BK virus VP1 protein by way of a linking sequence.

72. A method for enhancing an immune response in an animal comprising introducing into said animal a composition comprising: (a) a virus-like particle; and (b) an immunostimulatory substance; wherein said immunostimulatory substance is bound to said virus-like particle.

73. The method of claim 72, wherein said composition further comprises an antigen, wherein said antigen is bound to said virus-like particle.

74. The method of claim 72, wherein said immunostimulatory substance is a toll-like receptor activating substance.

75. The method of claim 72, wherein said immunostimulatory substance is a cytokine secretion inducing substance.

76. The method of claim 74, wherein said toll-like receptor activating substance is selected from the group consisting of, or alternatively consists essentially of: (a) immunostimulatory nucleic acids; (b) peptidoglycans; (c) lipopolysaccharides; (d) lipoteichonic acids; (e) imidazoquinoline compounds; (f) flagellines; (g) lipoproteins; (h) immunostimulatory organic molecules; (i) unmethylated CpG-containing **oligonucleotides**; and (j) any mixtures of at least one substance of (a), (b), (c), (d), (e), (f), (g), (h) and/or (i).

77. The method of claim 76, wherein said immunostimulatory nucleic acid is selected from the group consisting of, or alternatively consists essentially of: (a) ribonucleic acids; (b) deoxyribonucleic acids; (c) chimeric nucleic acids; and (d) any mixtures of at least one nucleic acid of (a), (b) and/or (c).

78. The method of claim 77, wherein said ribonucleic acid is poly-(I:C) or a derivative thereof.

79. The method of claim 77, wherein said deoxyribonucleic acid is selected from the group consisting of, or alternatively consists essentially of: (a) unmethylated CpG-containing **oligonucleotides**; and (b) **oligonucleotides** free of unmethylated CpG motifs.

an unmethylated CpG-containing **oligonucleotide**.

81. The method of claim 72, wherein said virus-like particle lacks a lipoprotein-containing envelope.

82. The method of claim 72, wherein said virus-like particle is a recombinant virus-like particle.

83. The method of claim 82, wherein said virus-like particle is selected from the group consisting of: (a) recombinant proteins of Hepatitis B virus; (b) recombinant proteins of measles virus; (c) recombinant proteins of Sinbis virus; (d) recombinant proteins of Rotavirus; (e) recombinant proteins of Foot-and-Mouth-Disease virus; (f) recombinant proteins of Retrovirus; (g) recombinant proteins of Norwalk virus; (h) recombinant proteins of human Papilloma virus; (i) recombinant proteins of BK virus; (j) recombinant proteins of bacteriophages; (k) recombinant proteins of RNA-phages; (l) recombinant proteins of Q β -phage; (m) recombinant proteins of GA-phage; (n) recombinant proteins of fr-phage; (o) recombinant proteins of AP 205-phage; (p) recombinant proteins of Ty; and (q) fragments of any of the recombinant proteins from (a) to (p).

84. The method of claim 83, wherein said virus-like particle is the Hepatitis B virus core protein.

85. The method of claim 83, wherein said virus-like particle is the BK virus VP 1 protein.

86. The method of claim 72, wherein said virus-like particle comprises recombinant proteins, or fragments thereof, of a RNA-phage.

87. The method of claim 86, wherein said RNA-phage is selected from the group consisting of: (a) bacteriophage Q β ; (b) bacteriophage R17; (c) bacteriophage fr; (d) bacteriophage GA; (e) bacteriophage SP; (f) bacteriophage MS2; (g) bacteriophage M11; (h) bacteriophage MX1; (i) bacteriophage NL95; (k) bacteriophage f2; (l) bacteriophage PP7; and (m) bacteriophage AP205.

88. The method of claim 72, wherein said virus-like particle comprises recombinant proteins, or fragments thereof, of RNA-phage Q β .

89. The method of claim 72, wherein said virus-like particle comprises recombinant proteins, or fragments thereof, of RNA-phage AP 205.

90. The method of claim 80, wherein said unmethylated CpG-containing **oligonucleotide** comprises the sequence: 5'X₁X₂CX₃X₄.su b.4 3' wherein X₁, X₂, X₃, and X₄ are any nucleotide.

91. The method of claim 90, wherein at least one of said nucleotide X₁, X₂, X₃, and X₄ has a phosphate backbone modification.

92. The method of claim 80, wherein said unmethylated CpG-containing **oligonucleotide** comprises, or alternatively consists essentially of, or alternatively consists of the sequence selected from the group consisting of: (a) TCCATGACGTTCTGAATAAT; (b) TCCATGACGTTCTGACGTT; (c) GGGGTCAACGTTGAGGGGG; (d) GGGGGGGGGGACGATCGGGGGGGGGG; and (e) "dsCyCpG-253" as described in Table 1.

93. The method of claim 92, wherein said unmethylated CpG-containing **oligonucleotide** contains one or more phosphorothioate modifications of the phosphate backbone or wherein each phosphate moiety of said phosphate backbone of said **oligonucleotide** is a phosphorothioate modification.

94. The method of claim 80, wherein said unmethylated CpG-containing **oligonucleotide** is palindromic.

95. The method of claim 94, wherein said palindromic unmethylated CpG-containing **oligonucleotide** comprises, or alternatively consists essentially of, or alternatively consists of the sequence GGGGTCAACGTTGAGGGGG.

96. The method of claim 95, wherein said palindromic unmethylated CpG-containing **oligonucleotide** contains one or more phosphorothioate modifications of the phosphate backbone or wherein each phosphate moiety of said phosphate backbone of said **oligonucleotide** is a phosphorothioate modification.

97. The method of claim 72, wherein said immunostimulatory substance is

98. The method of claim 72, wherein said virus-like particle is produced in a bacterial expression system.

99. The method of claim 72, wherein said virus-like particle is produced in a yeast expression system.

100. The method of claim 80, wherein said unmethylated CpG-containing **oligonucleotide** is non-covalently bound to said virus-like particle..

101. The method of claim 72, wherein said immunostimulatory substance is packaged, preferably enclosed by said virus-like particle.

102. The method of claim 80, wherein said unmethylated CpG-containing **oligonucleotide** is packaged, preferably enclosed by said virus-like particle.

103. The method of claim 76, wherein said immunostimulatory nucleic acid is bound to a virus-like particle site selected from the group consisting of an **oligonucleotide** binding site, a DNA binding site and a RNA binding site

104. The method of claim 80, wherein said unmethylated CpG-containing **oligonucleotide** is bound to a virus-like particle site selected from the group consisting of an **oligonucleotide** binding site, a DNA binding site and a RNA binding site.

105. The method of claim 104, wherein said **oligonucleotide** binding site is a non-naturally occurring **oligonucleotide** binding site.

106. The method of claim 104, wherein said virus-like particle site comprises an arginine-rich repeat.

107. The method of claim 76, wherein said immunostimulatory nucleic acid contains one or more phosphorothioate modifications of the phosphate backbone or wherein each phosphate moiety of said phosphate backbone of said **oligonucleotide** is a phosphorothioate modification.

108. The method of claim 80, wherein said unmethylated CpG-containing **oligonucleotide** contains one or more phosphorothioate modifications of the phosphate backbone or wherein each phosphate moiety of said phosphate backbone of said **oligonucleotide** is a phosphorothioate modification.

109. The method of claim 76, wherein said immunostimulatory nucleic acid, and preferably said unmethylated CpG-containing **oligonucleotide** comprises about 6 to about 100,000 nucleotides.

110. The method of claim 109, wherein said immunostimulatory nucleic acid, and preferably said unmethylated CpG-containing **oligonucleotide** comprises about 6 to about 2000 nucleotides.

111. The method of claim 110, wherein said immunostimulatory nucleic acid, and preferably said unmethylated CpG-containing **oligonucleotide** comprises about 20 to about 2000 nucleotides.

112. The method of claim 111, wherein said immunostimulatory nucleic acid, and preferably said unmethylated CpG-containing **oligonucleotide** comprises about 20 to about 300 nucleotides.

113. The method of claim 112, wherein said immunostimulatory nucleic acid, and preferably said unmethylated CpG-containing **oligonucleotide** comprises 20 to 100 nucleotides.

114. The method of claim 76, wherein said immunostimulatory nucleic acid, and preferably said unmethylated CpG-containing **oligonucleotide** comprises more than 100 to about 2000 nucleotides.

115. The method of claim 114, wherein said immunostimulatory nucleic acid, and preferably said unmethylated CpG-containing **oligonucleotide** comprises more than 100 to about 1000 nucleotides.

116. The method of claim 115, wherein said immunostimulatory nucleic acid, and preferably said unmethylated CpG-containing **oligonucleotide** comprises more than 100 to about 500 nucleotides.

117. The method of claim 76, wherein said immunostimulatory nucleic acid, and preferably said unmethylated CpG-containing **oligonucleotide** is a recombinant **oligonucleotide**.

118. The method of claim 76, wherein said immunostimulatory nucleic acid, and preferably said unmethylated CpG-containing **oligonucleotide**

119. The method of claim 76, wherein said immunostimulatory nucleic acid, and preferably said unmethylated CpG-containing **oligonucleotide** is a synthetic **oligonucleotide**.

120. The method of claim 76, wherein said immunostimulatory nucleic acid, and preferably said unmethylated CpG-containing **oligonucleotide** is a plasmid-derived **oligonucleotide**.

121. The method of claim 76, wherein said immunostimulatory nucleic acid, and preferably said unmethylated CpG-containing **oligonucleotide** is a single-stranded **oligonucleotide**.

122. The method of claim 76, wherein said immunostimulatory nucleic acid, and preferably said unmethylated CpG-containing **oligonucleotide** is a double-stranded **oligonucleotide**.

123. The method of claim 73, wherein said at least one antigen or antigenic determinant is bound to said virus-like particle by at least one covalent bond.

124. The method of claim 73, wherein said at least one antigen or antigenic determinant is bound to said virus-like particle by at least one covalent bond, and wherein said covalent bond is a non-peptide bond.

125. The method of claim 73, wherein said at least one antigen or antigenic determinant is fused to said virus-like particle.

126. The method of claim 73, wherein said antigen or antigenic determinant further comprises at least one second attachment site selected from the group consisting of: (a) an attachment site not naturally occurring with said antigen or antigenic determinant; and (b) an attachment site naturally occurring with said antigen or antigenic determinant.

127. The method of claim 73, further comprising an amino acid linker, wherein said amino acid linker comprises, or alternatively consists of, said second attachment site.

128. The method of claim 73, wherein said antigen is selected from the group consisting of: (a) polypeptides; (b) carbohydrates; (c) steroid hormones; and (d) organic molecules.

129. The method of claim 128, wherein said antigen is an organic molecule.

130. The method of claim 129, wherein said organic molecule is selected from the group consisting of: (a) codeine; (b) fentanyl; (c) heroin; (d) morphium; (e) amphetamine; (f) cocaine; (g) methylenedioxymethamphetamine; (h) methamphetamine; (i) methylphenidate; (j) nicotine; (k) LSD; (l) mescaline; (m) psilocybin; and (n) tetrahydrocannabinol.

131. The method of claim 73, wherein said antigen is a recombinant antigen.

132. The method of claim 73, wherein said antigen is derived from the group consisting of: (a) viruses; (b) bacteria; (c) parasites; (d) prions; (e) tumors; (f) self-molecules; (g) non-peptidic hapten molecules (h) allergens; and (i) hormones.

133. The method of claim 132, wherein said antigen is a tumor antigen.

134. The method of claim 133, wherein said tumor antigen is selected from the group consisting of: (a) Her2; (b) GD2; (c) EGF-R; (d) CEA; (e) CD52; (f) CD21; (g) human melanoma protein gp100; (h) human melanoma protein melan-A/MART-1; (i) tyrosinase; (j) NA17-A nt protein; (k) MAGE-3 protein; (l) p53 protein; (m) HPV16 E7 protein; and (n) antigenic fragments of any of the tumor antigens from (a) to (m).

135. The method of claim 73, wherein said antigen is bound to said virus-like particle by way of a linking sequence.

136. The method of claim 135, wherein said virus-like particle is the Hepatitis B virus core protein.

137. The method of claim 135, wherein said virus-like particle is the BK virus VP1 protein.

138. The method of claim 73, wherein said antigen is a cytotoxic T cell epitope, a Th cell epitope or a combination of at least two of said

way of a linking sequence.

139. The method of claim 138, wherein said cytotoxic T cell epitope is a viral or a tumor cytotoxic T cell epitope.

140. The method of claim 138, wherein said antigen is bound to said virus-like particle by way of a linking sequence.

141. The method of claim 138, wherein said virus-like particle is the Hepatitis B virus core protein.

142. The method of claim 141, wherein said cytotoxic T cell epitope is fused to the C-terminus of said Hepatitis B virus core protein.

143. The method of claim 142, wherein said cytotoxic T cell epitope is fused to the C-terminus of said Hepatitis B virus core protein by way of a linking sequence.

144. The method of claim 138, wherein said virus-like particle is a BK virus VPI protein.

145. The method of claim 144, wherein said cytotoxic T cell epitope is fused to the C-terminus of said BK virus VPI protein.

146. The method of claim 145, wherein said cytotoxic T cell epitope is fused to the C-terminus of said BK virus VPI protein by way of a linking sequence.

147. The method of claim 72, wherein said immune response is an enhanced B cell response.

148. The method of claim 72, wherein said immune response is an enhanced T cell response.

149. The method of claim 148, wherein said T cell response is a CTL response.

150. The method of claim 148, wherein said T cell response is a Th cell response.

151. The method of claim 150, wherein said Th cell response is a Th1 cell response.

152. The method of claim 72, wherein said animal is a mammal.

153. The method of claim 152, wherein said mammal is a human.

154. The method of claim 72, wherein said composition is introduced into said animal subcutaneously, intramuscularly, intravenously, intranasally or directly into the lymph node.

155. A method of producing a composition for enhancing an immune response in an animal comprising a virus-like particle and an immunostimulatory substance bound to said virus-like particle which comprises: (a) incubating said virus-like particle with said immunostimulatory substance; (b) adding RNase; and (c) purifying said composition.

156. The method of claim 155, wherein said immunostimulatory substance is an immunostimulatory nucleic acid selected from the group consisting of, or alternatively consists essentially of: (a) ribonucleic acids; (b) deoxyribonucleic acids; (c) chimeric nucleic acids; and (d) any mixtures of at least one nucleic acid of (a), (b) and/or (c).

157. The method of claim 156, wherein said ribonucleic acid is poly-(I:C) or a derivative thereof.

158. The method of claim 156, wherein said deoxyribonucleic acid is selected from the group consisting of, or alternatively consists essentially of: (a) unmethylated CpG-containing **oligonucleotides**; and (b) **oligonucleotides** free of unmethylated CpG motifs.

159. The method of claim 155, wherein said immunostimulatory substance is an unmethylated CpG-containing **oligonucleotide**.

160. The method of claim 155, wherein said virus-like particle is produced in a bacterial expression system.

161. The method of claim 155, wherein said RNase is RNase A.

162. A method of producing a composition for enhancing an immune response in an animal comprising a virus-like particle and an

comprises: (a) incubating said virus-like particle with RNase; (b) adding said immunostimulatory substance; and (c) purifying said composition.

163. The method of claim 162, wherein said immunostimulatory substance is an immunostimulatory nucleic acid selected from the group consisting of, or alternatively consists essentially of: (a) ribonucleic acids; (b) deoxyribonucleic acids; (c) chimeric nucleic acids; and (d) any mixtures of at least one nucleic acid of (a), (b) and/or (c).

164. The method of claim 163, wherein said ribonucleic acid is poly-(I:C) or a derivative thereof.

165. The method of claim 163, wherein said deoxyribonucleic acid is selected from the group consisting of, or alternatively consists essentially of: (a) unmethylated CpG-containing **oligonucleotides**; and (b) **oligonucleotides** free of unmethylated CpG motifs.

166. The method of claim 162, wherein said immunostimulatory substance is an unmethylated CpG-containing **oligonucleotide**.

167. The method of claim 162, wherein said virus-like particle is produced in a bacterial expression system.

168. The method of claim 162, wherein said RNase is RNase A.

169. A method of producing a composition for enhancing an immune response in an animal comprising a virus-like particle and an immunostimulatory substance bound to said virus-like particle which comprises: (a) disassembling said virus-like particle; (b) adding said immunostimulatory substance; and (c) reassembling said virus-like particle.

170. The method of claim 169, wherein said immunostimulatory substance is an immunostimulatory nucleic acid selected from the group consisting of, or alternatively consists essentially of: (a) ribonucleic acids; (b) deoxyribonucleic acids; (c) chimeric nucleic acids; and (d) any mixtures of at least one nucleic acid of (a), (b) and/or (c).

171. The method of claim 170, wherein said ribonucleic acid is poly-(I:C) or a derivative thereof.

172. The method of claim 170, wherein said deoxyribonucleic acid is selected from the group consisting of, or alternatively consists essentially of: (a) unmethylated CpG-containing **oligonucleotides**; and (b) **oligonucleotides** free of unmethylated CpG motifs.

173. The method of claim 169, wherein said immunostimulatory substance is an unmethylated CpG-containing **oligonucleotide**.

174. The method of claim 169 further comprising removing nucleic acids of said disassembled virus-like particle.

175. The method of claim 169 further comprising purifying said composition after reassembly (c).

176. A method of producing a composition for enhancing an immune response in an animal comprising a virus-like particle and an immunostimulatory substance bound to said virus-like particle which comprises: (a) incubating said virus-like particle with solutions comprising metal ions capable of hydrolyzing the nucleic acids of said virus-like particle; (b) adding said immunostimulatory substance; and (c) purifying said composition.

177. The method of claim 176, wherein said immunostimulatory substance is an immunostimulatory nucleic acid selected from the group consisting of, or alternatively consists essentially of: (a) ribonucleic acids; (b) deoxyribonucleic acids; (c) chimeric nucleic acids; and (d) any mixtures of at least one nucleic acid of (a), (b) and/or (c).

178. The method of claim 177, wherein said ribonucleic acid is poly-(I:C) or a derivative thereof.

179. The method of claim 177, wherein said deoxyribonucleic acid is selected from the group consisting of, or alternatively consists essentially of: (a) unmethylated CpG-containing **oligonucleotides**; and (b) **oligonucleotides** free of unmethylated CpG motifs.

180. The method of claim 176, wherein said immunostimulatory substance is an unmethylated CpG-containing **oligonucleotide**.

181. The method of claim 176, wherein said metal ions are selected from

(c) iron (Fe) ions; and (d) any mixtures of at least one ion of (a), (b) and/or (c).

182. A vaccine comprising an immunologically effective amount of the composition of claim 1 together with a pharmaceutically acceptable diluent, carrier or excipient.

183. The vaccine of claim 182 further comprising an adjuvant.

184. A method of immunizing or treating an animal comprising administering to said animal an immunologically effective amount of the vaccine of claim 182.

185. The method of claim 184, wherein said animal is a mammal.

186. The method of claim 185, wherein said mammal is a human.

187. A vaccine comprising an immunologically effective amount of the composition of claim 2 together with a pharmaceutically acceptable diluent, carrier or excipient.

188. The vaccine of claim 187 further comprising an adjuvant.

189. A method of immunizing or treating an animal comprising administering to said animal an immunologically effective amount of the vaccine of claim 187.

190. The method of claim 189, wherein said animal is a mammal.

191. The method of claim 190, wherein said mammal is a human.

192. A method of immunizing or treating an animal comprising priming a T cell response in said animal by administering an immunologically effective amount of the vaccine of claim 182.

193. The method of claim 192, further comprising the step of boosting the immune response in said animal.

194. The method of claim 193, wherein said boosting is effected by administering an immunologically effective amount of a vaccine of claim 182 or an immunologically effective amount of a heterologous vaccine.

195. The method of claim 194, wherein said heterologous vaccine is a DNA vaccine.

196. A method of immunizing or treating an animal comprising boosting a T cell response in said animal by administering an immunologically effective amount of the vaccine of claim 182.

197. The method of claim 196, further comprising the step of priming a T cell response in said animal.

198. The method of claim 197, wherein said priming is effected by administering an immunologically effective amount of a vaccine of claim 182 or an immunologically effective amount of a heterologous vaccine.

199. The method of claim 198, wherein said heterologous vaccine is a DNA vaccine.

200. A method of immunizing or treating an animal comprising priming a T cell response in said animal by administering an immunologically effective amount of the vaccine of claim 187.

201. The method of claim 200 further comprising the step of boosting the immune response in said animal.

202. The method of claim 201, wherein said boosting is effected by administering an immunologically effective amount of a vaccine of claim 187 or an immunologically effective amount of a heterologous vaccine.

203. The method of claim 202, wherein said heterologous vaccine is a DNA vaccine or a viral vaccine or a canary pox vaccine.

204. A method of immunizing or treating an animal comprising boosting a T cell response in said animal by administering an immunologically effective amount of the vaccine of claim 187.

205. The method of claim 204, further comprising the step of priming a T cell response in said animal.

206. The method of claim 205, wherein said priming is effected by administering an immunologically effective amount of a vaccine of claim

207. The method of claim 206, wherein said heterologous vaccine is a DNA vaccine or a viral vaccine or a canary pox vaccine.

L5 ANSWER 54 OF 112 USPTAFULL on STN

2003:140406 Human cDNAs and proteins and uses thereof.

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GENSET, S.A., Paris, FRANCE, 75008 (non-U.S. corporation)

US 2003096247 A1 20030522

APPLICATION: US 2001-986 A1 20011114 (10)

PRIORITY: WO 2001-IB1715 20010806

US 2001-305456P 20010713 (60)

US 2001-302277P 20010629 (60)

US 2001-298698P 20010615 (60)

US 2001-293574P 20010525 (60)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention concerns GENSET polynucleotides and polypeptides. Such GENSET products may be used as reagents in forensic analyses, as chromosome markers, as tissue/cell/organelle-specific markers, in the production of expression vectors. In addition, they may be used in screening and diagnosis assays for abnormal GENSET expression and/or biological activity and for screening compounds that may be used in the treatment of GENSET-related disorders.

CLM What is claimed is:

1. An isolated polynucleotide, comprising a nucleic acid sequence selected from the group consisting of: a) a polynucleotide of SEQ ID NO:19, or of a human cDNA of deposited clone 106-030-2-0-A3-F, encoding at least any single integer from 6 to 500 amino acids of SEQ ID NO:20; b) a polynucleotide of SEQ ID NO: 19, or of a human cDNA of deposited clone 106-030-2-0-A3-F, encoding the signal peptide sequence of SEQ ID NO:20; c) a polynucleotide of SEQ ID NO: 19, or of a human cDNA of deposited clone 106-030-2-0-A3-F, encoding a mature polypeptide sequence of SEQ ID NO:20; d) a polynucleotide of SEQ ID NO: 19, or of a human cDNA of deposited clone 106-030-2-0-A3-F, encoding a full length polypeptide sequence of SEQ ID NO:20; e) a polynucleotide of SEQ ID NO: 19, or of a human cDNA of deposited clone 106-030-2-0-A3-F, encoding a polypeptide sequence of a biologically active fragment of SEQ ID NO:20; f) a polynucleotide encoding a polypeptide sequence of at least any single integer from 6 to 500 amino acids of SEQ ID NO:20 or of a polypeptide encoded by a human cDNA of deposited clone 106-030-2-0-A3-F; g) a polynucleotide encoding a polypeptide sequence of a signal peptide of SEQ ID NO:20 or of a signal peptide encoded by a human cDNA of deposited clone 106-030-2-0-A3-F; h) a polynucleotide encoding a polypeptide sequence of a mature polypeptide of SEQ ID NO:20 or of a mature polypeptide encoded by a human cDNA of deposited clone 106-030-2-0-A3-F; i) a polynucleotide encoding a polypeptide sequence of a full length polypeptide of SEQ ID NO:20 or of a mature polypeptide encoded by a human cDNA of deposited clone 106-030-2-0-A3-F; j) a polynucleotide encoding a polypeptide sequence of a biologically active polypeptide of SEQ ID NO:20, or of a biologically active polypeptide encoded by a human cDNA of deposited clone 106-030-2-0-A3-F; k) a polynucleotide of any one of a) through j) further comprising an expression vector; l) a host cell recombinant for a polynucleotide of a) through k) above; m) a non-human transgenic animal comprising the host cell of k); and n) a polynucleotide of a) through j) further comprising a physiologically acceptable carrier.

2. A polypeptide comprising an amino acid sequence selected from the group consisting of: a) any single integer from 6 to 500 amino acids of SEQ ID NO:20 or of a polypeptide encoded by a human cDNA of deposited clone 106-030-2-0-A3-F; b) a signal peptide sequence of SEQ ID NO:20 or encoded by a human cDNA of deposited clone 106-030-2-0-A3-F; c) a mature polypeptide sequence of SEQ ID NO:20 or encoded by a human cDNA of deposited clone 106-030-2-0-A3-F; d) a full length polypeptide sequence of SEQ ID NO:20 or encoded by a human cDNA of deposited clone 106-030-2-0-A3-F; and e) a polypeptide of a) through d) further comprising a physiologically acceptable carrier.

3. A method of making a polypeptide, said method comprising: a) providing a population of host cells comprising the polynucleotide of claim 1; b) culturing said population of host cells under conditions conducive to the production of a polypeptide of claim 2 within said host cells; and c) purifying said polypeptide from said population of host cells.

4. A method of making a polypeptide, said method comprising: a) providing a population of cells comprising a polynucleotide encoding the polypeptide of claim 2, operably linked to a promoter; b) culturing said population of cells under conditions conducive to the production of

from said population of cells.

5. An antibody that specifically binds to the polypeptide of claim 2.

6. A method of binding a polypeptide of claim 2 to an antibody of claim 5, comprising contacting said antibody with said polypeptide under conditions in which antibody can specifically bind to said polypeptide.

7. A method of determining whether a OAR gene is expressed within a mammal, said method comprising the steps of: a) providing a biological sample from said mammal; b) contacting said biological sample with either of: i) a polynucleotide that hybridizes under stringent conditions to the polynucleotide of claim 1; or ii) a polypeptide that specifically binds to the polypeptide of claim 2; and c) detecting the presence or absence of hybridization between said polynucleotide and an RNA species within said sample, or the presence or absence of binding of said polypeptide to a protein within said sample; wherein a detection of said hybridization or of said binding indicates that said OAR gene is expressed within said mammal.

8. The method of claim 7, wherein said polynucleotide is a **primer**, and wherein said hybridization is detected by detecting the presence of an amplification product comprising the sequence of said **primer**.

9. The method of claim 7, wherein said polypeptide is an antibody.

10. A method of determining whether a mammal has an elevated or reduced level of a OAR gene expression, said method comprising the steps of: a) providing a biological sample from said mammal; and b) comparing the amount of the polypeptide of claim 2, or of an RNA species encoding said polypeptide, within said biological sample with a level detected in or expected from a control sample; wherein an increased amount of said polypeptide or said RNA species within said biological sample compared to said level detected in or expected from said control sample indicates that said mammal has an elevated level of said OAR gene expression, and wherein a decreased amount of said polypeptide or said RNA species within said biological sample compared to said level detected in or expected from said control sample indicates that said mammal has a reduced level of said OAR gene expression.

11. A method of identifying a candidate modulator of a OAR polypeptide, said method comprising: a) contacting the polypeptide of claim 2 with a test compound; and b) determining whether said compound specifically binds to said polypeptide; wherein a detection that said compound specifically binds to said polypeptide indicates that said compound is a candidate modulator of said OAR polypeptide.

12. The method of claim 11, further comprising testing the biological activity of said OAR polypeptide in the presence of said candidate modulator, wherein an alteration in the biological activity of said OAR polypeptide in the presence of said compound in comparison to the activity in the absence of said compound indicates that the compound is a modulator of said OAR polypeptide.

13. A method for the production of a pharmaceutical composition comprising a) identifying a modulator of a OAR polypeptide using the method of claim 11; and b) combining said modulator with a physiologically acceptable carrier.

L5 ANSWER 55 OF 112 USPTAFULL on STN

2003:133926 Human cDNAs and proteins and uses thereof.

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GENSET, S.A., Paris, FRANCE, 75008 (non-U.S. corporation)

US 2003092011 A1 20030515

APPLICATION: US 2001-489 A1 20011114 (10)

PRIORITY: WO 2001-IB1715 20010806

US 2001-305456P 20010713 (60)

US 2001-302277P 20010629 (60)

US 2001-298698P 20010615 (60)

US 2001-293574P 20010525 (60)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention concerns GENSET polynucleotides and polypeptides. Such GENSET products may be used as reagents in forensic analyses, as chromosome markers, as tissue/cell/organelle-specific markers, in the production of expression vectors. In addition, they may be used in screening and diagnosis assays for abnormal GENSET expression and/or biological activity and for screening compounds that may be used in the treatment of GENSET-related disorders.

CLM What is claimed is:

selected from the group consisting of: a) a polynucleotide of SEQ ID NO:23, or of a human cDNA of deposited clone 47-14-1-C3-CL0--5, encoding at least any single integer from 6 to 500 amino acids of SEQ ID NO:24; b) a polynucleotide of SEQ ID NO:23, or of a human cDNA of deposited clone 47-14-1-C3-CL0--5, encoding the signal peptide sequence of SEQ ID NO:24; c) a polynucleotide of SEQ ID NO:23, or of a human cDNA of deposited clone 47-14-1-C3-CL0--5, encoding a mature polypeptide sequence of SEQ ID NO:24; d) a polynucleotide of SEQ ID NO:23, or of a human cDNA of deposited clone 47-14-1-C3-CL0--5, encoding a full length polypeptide sequence of SEQ ID NO:24; e) a polynucleotide of SEQ ID NO:23, or of a human cDNA of deposited clone 47-14-1-C3-CL0--5, encoding a polypeptide sequence of a biologically active fragment of SEQ ID NO:24; f) a polynucleotide encoding a polypeptide sequence of at least any single integer from 6 to 500 amino acids of SEQ ID NO:24 or of a polypeptide encoded by a human cDNA of deposited clone 47-14-1-C3-CL0--5; g) a polynucleotide encoding a polypeptide sequence of a signal peptide of SEQ ID NO:24 or of a signal peptide encoded by a human cDNA of deposited clone 47-14-1-C3-CL0--5; h) a polynucleotide encoding a polypeptide sequence of a mature polypeptide of SEQ ID NO:24 or of a mature polypeptide encoded by a human cDNA of deposited clone 47-14-1-C3-CL0--5; i) a polynucleotide encoding a polypeptide sequence of a full length polypeptide of SEQ ID NO:24 or of a mature polypeptide encoded by a human cDNA of deposited clone 47-14-1-C3-CL0--5; j) a polynucleotide encoding a polypeptide sequence of a biologically active polypeptide of SEQ ID NO:24, or of a biologically active polypeptide encoded by a human cDNA of deposited clone 47-14-1-C3-CL0--5; k) a polynucleotide of any one of a) through j) further comprising an expression vector; l) a host cell recombinant for a polynucleotide of a) through k) above; m) a non-human transgenic animal comprising the host cell of k); and n) a polynucleotide of a) through j) further comprising a physiologically acceptable carrier.

2. A polypeptide comprising an amino acid sequence selected from the group consisting of: a) any single integer from 6 to 500 amino acids of SEQ ID NO:24 or of a polypeptide encoded by a human cDNA of deposited clone 47-14-1-C3-CL0--5; b) a signal peptide sequence of SEQ ID NO:24 or encoded by a human cDNA of deposited clone 47-14-1-C3-CL0--5; c) a mature polypeptide sequence of SEQ ID NO:24 or encoded by a human cDNA of deposited clone 47-14-1-C3-CL0--5; d) a full length polypeptide sequence of SEQ ID NO:24 or encoded by a human cDNA of deposited clone 47-14-1-C3-CL0--5; and e) a polypeptide of a) through d) further comprising a physiologically acceptable carrier.

3. A method of making a polypeptide, said method comprising: a) providing a population of host cells comprising the polynucleotide of claim 1; b) culturing said population of host cells under conditions conducive to the production of a polypeptide of claim 2 within said host cells; and c) purifying said polypeptide from said population of host cells.

4. A method of making a polypeptide, said method comprising: a) providing a population of cells comprising a polynucleotide encoding the polypeptide of claim 2, operably linked to a promoter; b) culturing said population of cells under conditions conducive to the production of said polypeptide within said cells; and c) purifying said polypeptide from said population of cells.

5. An antibody that specifically binds to the polypeptide of claim 2.

6. A method of binding a polypeptide of claim 2 to an antibody of claim 5, comprising contacting said antibody with said polypeptide under conditions in which antibody can specifically bind to said polypeptide.

7. A method of determining whether a APIP gene is expressed within a mammal, said method comprising the steps of: a) providing a biological sample from said mammal; b) contacting said biological sample with either of: i) a polynucleotide that hybridizes under stringent conditions to the polynucleotide of claim 1; or ii) a polypeptide that specifically binds to the polypeptide of claim 2; and c) detecting the presence or absence of hybridization between said polynucleotide and an RNA species within said sample, or the presence or absence of binding of said polypeptide to a protein within said sample; wherein a detection of said hybridization or of said binding indicates that said APIP gene is expressed within said mammal.

8. The method of claim 7, wherein said polynucleotide is a **primer**, and wherein said hybridization is detected by detecting the presence of an amplification product comprising the sequence of said **primer**.

10. A method of determining whether a mammal has an elevated or reduced level of a APIP gene expression, said method comprising the steps of: a) providing a biological sample from said mammal; and b) comparing the amount of the polypeptide of claim 2, or of an RNA species encoding said polypeptide, within said biological sample with a level detected in or expected from a control sample; wherein an increased amount of said polypeptide or said RNA species within said biological sample compared to said level detected in or expected from said control sample indicates that said mammal has an elevated level of said APIP gene expression, and wherein a decreased amount of said polypeptide or said RNA species within said biological sample compared to said level detected in or expected from said control sample indicates that said mammal has a reduced level of said APIP gene expression.

11. A method of identifying a candidate modulator of a APIP polypeptide, said method comprising: a) contacting the polypeptide of claim 2 with a test compound; and b) determining whether said compound specifically binds to said polypeptide; wherein a detection that said compound specifically binds to said polypeptide indicates that said compound is a candidate modulator of said APIP polypeptide.

12. The method of claim 11, further comprising testing the biological activity of said APIP polypeptide in the presence of said candidate modulator, wherein an alteration in the biological activity of said APIP polypeptide in the presence of said compound in comparison to the activity in the absence of said compound indicates that the compound is a modulator of said APIP polypeptide.

13. A method for the production of a pharmaceutical composition comprising a) identifying a modulator of a APIP polypeptide using the method of claim 11; and b) combining said modulator with a physiologically acceptable carrier.

L5 ANSWER 56 OF 112 USPTAFULL on STN
2003:133906 Gene detecting method and test kit.

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US 2003091991 A1 20030515

APPLICATION: US 2002-291028 A1 20021108 (10)

PRIORITY: JP 2001-345133 20011109

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A test kit that enables to rapidly specify a true pathogen among a plurality of pathogen candidates suspected of having caused infectious disease. The test kit includes a primer reagent obtained by mixing 10 to 100 kinds of amplifying primers, and a DNA microarray having spots of DNAs having a base sequence to be amplified by each of the amplifying primers.

CLM What is claimed is:

1. A method for determining whether a polynucleotide having a target base sequence is present in a test sample, comprising the steps of: preparing a gene detecting **primer** reagent by mixing at least ten kinds of amplifying **primers**; preparing a DNA microarray by spotting DNAs having a base sequence to be amplified by the amplifying **primers**, respectively, or DNAs substantially identical to the DNAs on a substrate; extracting a sample extract from the test sample; amplifying a gene by performing the **PCR** reaction using the sample extract and the gene detecting **primer** reagent; and detecting the **PCR** reaction product using the DNA microarray.

2. The method according to claim 1, wherein the step of preparing the gene detecting **primer** reagent includes a step of mixing 10 to 100 kinds of amplifying **primers**.

3. The method according to claim 1, wherein the test sample is selected from a group consisting of humor, living body tissue, living body secreta, living body excrement, food, drink, soil, ground water, seawater and lake marsh water, and wherein the step of preparing the gene detecting **primer** reagent includes a step of selecting a amplifying **primer** for amplifying a base sequence inherent to a pathogen of infectious disease or food poisoning, as one of the at least ten kinds of amplifying **primers**.

4. The method according to claim 3, wherein the step of preparing the gene detecting **primer** reagent includes a step of selecting a amplifying **primer** for amplifying another base sequence inherent to the pathogen, as one of the at least ten kinds of amplifying **primers**.

5. The method according to claim 3, wherein the step of preparing the gene detecting **primer** reagent includes a step of selecting a

plurality of pathogens belonging to a predetermined pathogen group corresponding to a predetermined test purpose, among a plurality of pathogen groups corresponding to a plurality of test purposes, respectively, as one of the at least ten kinds of amplifying **primers**, and wherein the pathogens are classified into a plurality of groups depending on the symptom and the infection route.

6. The method according to claim 1, wherein the test sample is human living body tissue, and the DNA is a gene for gene diagnosis or a fragment thereof.

7. The method according to claim 6, wherein the gene used for gene diagnosis is one of an oncogene and a gene for single base polymorphism diagnosis.

8. The method according to claim 1, wherein the target base sequence is one of a base sequence encoding a gene essential for the existence of a pathogen and a gene fragment thereof.

9. The method according to claim 1, wherein the gene amplifying step includes performing the **PCR** reaction by adding a labeling nucleotide.

10. A gene detecting **primer** reagent for detecting a polynucleotide having a target base sequence in a test sample, wherein the reagent comprises a mixture of at least ten kinds of amplifying **primers**.

11. A DNA microarray for detecting a polynucleotide having a target base sequence in a test sample, comprising: a substrate; and spots of DNAs having a base sequence to be amplified by each of 10 or more kinds of amplifying **primers**, or of DNAs substantially identical to the DNAs, formed on the substrate.

12. A test kit for detecting a polynucleotide having a target base sequence in a test sample, comprising: a **primer** reagent obtained by mixing at least ten kinds of amplifying **primers**; and a DNA microarray having spots of DNAs having a base sequence to be amplified by each of the amplifying **primers**, or of DNAs substantially identical to the DNAs.

13. A method of manufacturing a test kit for detecting a polynucleotide having a target base sequence in a test sample, comprising the steps of: preparing a gene detecting **primer** reagent by mixing at least ten kinds of amplifying **primers**; and preparing a DNA microarray by spotting DNAs having a base sequence to be amplified by the amplifying **primers**, or DNAs substantially identical to the DNAs on a substrate.

14. A test kit for specifying a pathogen of infectious disease, which comprising: a **primer** reagent obtained by mixing a plurality of amplifying **primers** for amplifying a base sequence inherent to a plurality of pathogens causing similar symptom to symptom of the infectious disease, respectively; and a DNA microarray having DNA spots having a polynucleotide inherent to the plurality of pathogens.

15. The test kit according to claim 14, wherein the plurality of amplifying **primers** comprise at least ten **primer** pairs.

16. The test kit according to claim 15, wherein the plurality of DNA spots comprise spots of at least ten kinds of target DNAs.

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2003:133508 In vivo activation of antigen presenting cells for enhancement of immune responses induced by virus like particles.

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US: 2003091593 A1 20030515

APPLICATION: US 2002-243739 A1 20020916 (10)

PRIORITY: US 2001-318967P 20010914 (60)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention relates to the finding that stimulation of antigen presenting cell (APC) activation using substances such as anti-CD40 antibodies or DNA oligomers rich in non-methylated C and G (CpGs) can dramatically enhance the specific T cell response obtained after vaccination with recombinant virus like particles (VLPs) coupled, fused or otherwise attached to antigens. While vaccination with recombinant VLPs fused to a cytotoxic T cell (CTL) epitope of lymphocytic choriomeningitis virus induced low levels cytolytic activity only and did not induce efficient anti-viral protection, VLPs injected together with anti-CD40 antibodies or CpGs induced strong CTL activity and full anti-viral protection. Thus, stimulation of APC-activation through

can exhibit a potent adjuvant effect for vaccination with VLPs coupled, fused or attached otherwise to antigens.

What is claimed is:

1. A composition for enhancing an immune response against an antigen in an animal comprising: (a) a virus-like particle bound to at least one antigen capable of inducing an immune response against said antigen in said animal; and (b) at least one substance that activates antigen presenting cells in an amount sufficient to enhance the immune response of said animal to said antigen.

2. The composition of claim 1, wherein said virus-like particle (a) lacks a lipoprotein-containing envelope.

3. The composition of claim 1, wherein said virus-like particle (a) is a recombinant virus-like particle.

4. The composition of claim 3, wherein said virus-like particle is selected from the group consisting of: (a) recombinant proteins of Hepatitis B virus; (b) recombinant proteins of measles virus; (c) recombinant proteins of Sindbis virus; (d) recombinant proteins of Rotavirus; (e) recombinant proteins of Foot-and-Mouth-Disease virus; (f) recombinant proteins of Retrovirus; (g) recombinant proteins of Norwalk virus; (h) recombinant proteins of human Papilloma virus; (i) recombinant proteins of BK virus; (j) recombinant proteins of bacteriophages; (k) recombinant proteins of RNA-phages; (l) recombinant proteins of Q β -phage; (m) recombinant proteins of GA-phage; (n) recombinant proteins of fr-phage; (o) recombinant proteins of AP 205-phage; (p) recombinant proteins of Ty; and (q) fragments of any of the recombinant proteins from (a) to (p).

5. The composition of claim 4, wherein said virus-like particle is the Hepatitis B virus core protein.

6. The composition of claim 1, wherein said antigen (a) is a recombinant antigen.

7. The composition of claim 1, wherein said antigen (a) is bound to said virus-like particle by way of a linking sequence.

8. The composition of claim 7, wherein said linking sequence comprises a sequence recognized by the proteasome, endosomal proteases or a protease contained in any other vesicular compartment of said antigen presenting cells.

9. The composition of claim 7, wherein said virus-like particle is the Hepatitis B virus core protein.

10. The composition of claim 1, wherein said antigen (a) is a cytotoxic T cell epitope, a Th cell epitope or a combination of at least two of said epitopes, wherein said at least two epitopes are linked directly or by way of a linking sequence.

11. The composition of claim 10, wherein said cytotoxic T cell epitope is a viral or a tumor cytotoxic T cell epitope.

12. The composition of claim 10, wherein said antigen is bound to said virus-like particle by way of a linking sequence

13. The composition of claim 10, wherein said virus-like particle is the Hepatitis B virus core protein.

14. The composition of claim 13, wherein said cytotoxic T cell epitope is fused to the C-terminus of said Hepatitis B virus core protein.

15. The composition of claim 14, wherein said cytotoxic T cell epitope is fused to the C-terminus of said Hepatitis B virus core protein by way of a linking sequence.

16. The composition of claim 1, wherein said virus-like particle (a) bound to said antigen has the amino acid sequence shown in FIG. 1.

17. The composition of claim 1, wherein said antigen (a) is selected from the group consisting of: (a) polypeptides; (b) carbohydrates; (c) steroid hormones; and (d) organic molecules.

18. The composition of claim 17, wherein said antigen is an organic molecule.

19. The composition of claim 18, wherein said organic molecule is selected from the group consisting of: (a) codeine; (b) fentanyl; (c) heroin; (d) morphium; (e) amphetamine; (f) cocaine; (g) methylenedioxymethamphetamine; (h) methamphetamine; (i)

psilocybin; and (n) tetrahydrocannabinol.

20. The composition of claim 1, wherein said antigen (a) is derived from the group consisting of: (a) viruses; (b) bacteria; (c) parasites; (d) prions; (e) tumors; (f) self-molecules; (g) non-peptidic hapten molecules; and (h) allergens.

21. The composition of claim 20, wherein said antigen is a tumor antigen.

22. The composition of claim 21, wherein said tumor antigen is selected from the group consisting of: (a) Her2; (b) GD2; (c) EGF-R; (d) CEA; (e) CD52; (f) CD21; (g) human melanoma protein gp100; (h) human melanoma protein melan-A/MART-1; (i) tyrosinase; (j) NA17-A nt protein; (k) MAGE-3 protein; (l) p53 protein; (m) HPV16 E7 protein; and (n) antigenic fragments of any of tumor antigens (a) to (m).

23. The composition of claim 1, wherein said virus-like particle comprises recombinant proteins, or fragments thereof, of a RNA-phage.

24. The composition of claim 23, wherein said RNA-phage is selected from the group consisting of: (a) bacteriophage Q β ; (b) bacteriophage R17; (c) bacteriophage fr; (d) bacteriophage GA; (e) bacteriophage SP; (f) bacteriophage MS2; (g) bacteriophage M11; (h) bacteriophage MX1; (i) bacteriophage NL95; (k) bacteriophage f2; (l) bacteriophage PP7; and (m) bacteriophage AP205.

25. The composition of claim 1, wherein said virus-like particle comprises recombinant proteins, or fragments thereof, of RNA-phage Q β .

26. The composition of claim 1, wherein said virus-like particle comprises recombinant proteins, or fragments thereof, of RNA-phage AP 205.

27. The composition of claim 1, wherein said substance (b) stimulates upregulation of costimulatory molecules on antigen presenting cells or secretion of cytokines.

28. The composition of claim 1, wherein said substance (b) induces nuclear translocation of NF-KB in antigen presenting cells.

29. The composition of claim 1, wherein said substance (b) activates toll-like receptors in antigen presenting cells.

30. The composition of claim 29, wherein said toll-like receptor activating substance is selected from the group consisting of, or alternatively consists essentially of: (a) immunostimulatory nucleic acids; (b) peptidoglycans; (c) lipopolysaccharides; (d) lipoteichonic acids; (e) imidazoquinoline compounds; (o) flagellines; (g) lipoproteins; (h) immunostimulatory organic molecules; (i) unmethylated CpG-containing **oligonucleotides**; and (j) any mixtures of at least one substance of (a), (b), (c), (d), (e), (f), (g), (h) and/or (i).

31. The composition of claim 30, wherein said immunostimulatory nucleic acid is selected from the group consisting of, or alternatively consists essentially of: (a) ribonucleic acids; (b) deoxyribonucleic acids; (c) chimeric nucleic acids; and (d) any mixtures of at least one nucleic acid of (a), (b) and/or (c).

32. The composition of claim 31, wherein said ribonucleic acid is poly-(I:C) or a derivative thereof.

33. The composition of claim 31, wherein said deoxyribonucleic acid is selected from the group consisting of, or alternatively consists essentially of: (a) unmethylated CpG-containing **oligonucleotides**; and (b) **oligonucleotides** free of unmethylated CpG motifs.

34. The composition of claim 1, wherein said immunostimulatory substance is an unmethylated CpG-containing **oligonucleotide**.

35. The composition of claim 1, wherein said substance (b) is selected from the group consisting of an anti-CD40 antibody, an immunostimulatory nucleic acid, an unmethylated CpG-containing **oligonucleotide** capable of activating APCs, and a palindromic **oligonucleotide**.

36. The composition of claim 34, wherein said unmethylated CpG-containing **oligonucleotide** comprises the sequence: 5'X₁X₂CX₃X₄3' wherein X₁, X₂, X₃, and X₄ are any nucleotide.

from the group consisting of an anti-CD40 antibody, an immunostimulatory nucleic acid, an unmethylated CpG-containing **oligonucleotide** capable of activating APCs, and a palindromic **oligonucleotide**.

38. The composition of claim 28, wherein said substance (b) is selected from the group consisting of an anti-CD40 antibody, an immunostimulatory nucleic acid, an unmethylated CpG-containing **oligonucleotide** capable of activating APCs, and a palindromic **oligonucleotide**.

39. The composition of claim 29, wherein said substance (b) is selected from the group consisting of an anti-CD40 antibody, an immunostimulatory nucleic acid, an unmethylated CpG-containing **oligonucleotide** capable of activating APCs, and a palindromic **oligonucleotide**.

40. The composition of claim 36, wherein at least one of said nucleotides X₁, X₂, X₃, and X₄ has a phosphate backbone modification.

41. The composition of claim 34, wherein said unmethylated CpG-containing **oligonucleotide** comprises, or alternatively consists essentially of, or alternatively consists of the sequence selected from the group consisting of:

- (a) TCCATGACGTTCTGAATAAT;
- (b) TCCATGACGTTCTGACGTT;
- (c) GGGGTCAACGTTGAGGGGG;
- (d) ATTATTAGGAACGTCATGGA;
- (e) GGGGGGGGGGACGATCGTCGGGGGGGGG;
- (f) TCCATGACGTTCTGAATAATAAATGCATGTCAA
GACAGCAT;
- (g) TCCATGACGTTCTGAATAATTCCATGACGTT
CCTGAATAATTCCATGACGTTCTGAATAAT;
- (h) TCCATGACGTTCTGAATAATCGCGCGCGCGC
GCGC GCGCGCGCGCGCGCGCGCGCGCG; and
- (i) TCGTCGTTTTGTCGTTTTGTCGT.

42. The composition of claim 41, wherein said unmethylated CpG-containing **oligonucleotide** contains one or more phosphorothioate modifications of the phosphate backbone or wherein each phosphate moiety of said phosphate backbone of said **oligonucleotide** is a phosphorothioate modification.

43. The composition of claim 34, wherein said unmethylated CpG-containing **oligonucleotide** is palindromic.

44. The composition of claim 43, wherein said palindromic unmethylated CpG-containing **oligonucleotide** comprises, or alternatively consists essentially of, or alternatively consists of the sequence GGGGTCAACGTTGAGGGGG.

45. The composition of claim 44, wherein said palindromic unmethylated CpG-containing **oligonucleotide** contains one or more phosphorothioate modifications of the phosphate backbone or wherein each phosphate moiety of said phosphate backbone of said **oligonucleotide** is a phosphorothioate modification.

46. The composition of claim 33, wherein said **oligonucleotide** free of unmethylated CpG motifs comprises, or alternatively consists essentially of, or alternatively consists of the sequence GGTCTTTTGGTCTTGTCT.

47. The composition of claim 1, wherein said antigen presenting cell is a dendritic cell.

48. The composition of claim 1, wherein said at least one antigen or antigenic determinant is bound to said virus-like particle by at least one covalent bond, and wherein said covalent bond is a non-peptide bond.

49. The composition of claim 1, wherein said at least one antigen or antigenic determinant is fused to said virus-like particle.

50. The composition of claim 1, wherein said antigen or antigenic

selected from the group consisting of: (a) an attachment site not naturally occurring with said antigen or antigenic determinant; and (b) an attachment site naturally occurring with said antigen or antigenic determinant.

51. The composition of claim 1 further comprising an amino acid linker, wherein said amino acid linker comprises, or alternatively consists of, a second attachment site.

52. A composition for enhancing an immune response against a virus-like particle in an animal comprising: (a) a virus-like particle capable of being recognized by the immune system of said animal and inducing an immune response against said virus-like particle in said animal; and (b) at least one substance that activates antigen presenting cells in an amount sufficient to enhance the immune response of said animal to said virus-like particle.

53. The composition of claim 52, wherein said virus-like particle (a) lacks a lipoprotein-containing envelope.

54. The composition of claim 52, wherein said virus-like particle (a) is a recombinant virus-like particle.

55. The composition of claim 54, wherein said virus-like particle is selected from the group consisting of: (a) recombinant proteins of Hepatitis B virus; (b) recombinant proteins of measles virus; (c) recombinant proteins of Sindbis virus; (d) recombinant proteins of Rotavirus; (e) recombinant proteins of Foot-and-Mouth-Disease virus; (f) recombinant proteins of Retrovirus; (g) recombinant proteins of Norwalk virus; (h) recombinant proteins of human Papilloma virus; (i) recombinant proteins of BK virus; (o) recombinant proteins of bacteriophages; (k) recombinant proteins of RNA-phages; (l) recombinant proteins of Q β -phage; (m) recombinant proteins of GA-phage; (n) recombinant proteins of fr-phage; (o) recombinant proteins of AP 205-phage; (p) recombinant proteins of Ty; and (q) fragments of any of the recombinant proteins from (a) to (p).

56. The composition of claim 55, wherein said virus-like particle is the Hepatitis B virus core protein.

57. The composition of claim 52, wherein said substance (b) stimulates upregulation of costimulatory molecules on antigen presenting cells.

58. The composition of claim 52, wherein said substance (b) induces nuclear translocation of NF- κ B in antigen presenting cells.

59. The composition of claim 52, wherein said substance (b) activates toll-like receptors in antigen presenting cells.

60. The composition of claim 59, wherein said toll-like receptor activating substance is selected from the group consisting of, or alternatively consists essentially of: (a) immunostimulatory nucleic acids; (b) peptidoglycans; (c) lipopolysaccharides; (d) lipoteichoic acids; (e) imidazoquinoline compounds; (f) flagellines; (g) lipoproteins; (h) immunostimulatory organic molecules; (i) unmethylated CpG-containing **oligonucleotides**; and (j) any mixtures of at least one substance of (a), (b), (c), (d), (e), (f), (g), (h) and/or (i).

61. The composition of claim 60, wherein said immunostimulatory nucleic acid is selected from the group consisting of, or alternatively consists essentially of: (a) ribonucleic acids; (b) deoxyribonucleic acids; (c) chimeric nucleic acids; and (d) any mixtures of at least one nucleic acid of (a), (b) and/or (c).

62. The composition of claim 61, wherein said ribonucleic acid is poly-(I:C) or a derivative thereof.

63. The composition of claim 61, wherein said deoxyribonucleic acid is selected from the group consisting of, or alternatively consists essentially of: (a) unmethylated CpG-containing **oligonucleotides**; and (b) **oligonucleotides** free of unmethylated CpG motifs.

64. The composition of claim 1, wherein said immunostimulatory substance is an unmethylated CpG-containing **oligonucleotide**.

65. The composition of claim 52, wherein said substance (b) is selected from the group consisting of an anti-CD40 antibody, an immunostimulatory nucleic acid, an unmethylated CpG-containing **oligonucleotide** capable of activating APCs, and a palindromic **oligonucleotide**.

66. The composition of claim 64, wherein said unmethylated

5'X₁X₂CGX₃X₄3' wherein X₁, X₂, X₃,
and X₄ are any nucleotide.

67. The composition of claim 57, wherein said substance (b) is selected from the group consisting of an anti-CD40 antibody, an immunostimulatory nucleic acid, an unmethylated CpG-containing **oligonucleotide** capable of activating APCs, and a palindromic **oligonucleotide**.

68. The composition of claim 58, wherein said substance (b) is selected from the group consisting of an anti-CD40 antibody, an immunostimulatory nucleic acid, an unmethylated CpG-containing **oligonucleotide** capable of activating APCs, and a palindromic **oligonucleotide**.

69. The composition of claim 59, wherein said substance (b) is selected from the group consisting of an anti-CD40 antibody, an immunostimulatory nucleic acid, an unmethylated CpG-containing **oligonucleotide** capable of activating APCs, and a palindromic **oligonucleotide**.

70. The composition of claim 52, wherein said antigen presenting cell is a dendritic cell, NK cell, macrophage or B cell.

71. The composition of claim 66, wherein at least one of said nucleotides X₁, X₂, X₃, and X₄ has a phosphate backbone modification.

72. The composition of claim 64, wherein said unmethylated CpG-containing **oligonucleotide** comprises, or alternatively consists essentially of, or alternatively consists of the sequence selected from the group consisting of:

- (a) TCCATGACGTTCTGAATAAT;
- (b) TCCATGACGTTCTGACGTT;
- (c) GGGGTCAACGTTGAGGGGG;
- (d) ATTATTCAGGAACGTCATGGA;
- (e) GGGGGGGGGGACGATCGTCGGGGGGGGGG;
- (f) TCCATGACGTTCTGAATAATAAATGCATGTCAAA
GACAGCAT;
- (g) TCCATGACGTTCTGAATAATTCCATGACGTT
CCTGAATAATTCCATGACGTTCTGAATAAT;
- (h) TCCATGACGTTCTGAATAATCGCGCGCGCGC
GCGC GCGCGCGCGCGCGCGCGCGCGCG; and
- (i) TCGTCGTTTTGTCGTTTTGTCGT.

73. The composition of claim 72, wherein said unmethylated CpG-containing **oligonucleotide** contains one or more phosphorothioate modifications of the phosphate backbone or wherein each phosphate moiety of said phosphate backbone of said **oligonucleotide** is a phosphorothioate modification.

74. The composition of claim 64, wherein said unmethylated CpG-containing **oligonucleotide** is palindromic.

75. The composition of claim 74, wherein said palindromic unmethylated CpG-containing **oligonucleotide** comprises, or alternatively consists essentially of, or alternatively consists of the sequence GGGGTCAACGTTGAGGGGG.

76. The composition of claim 75, wherein said palindromic unmethylated CpG-containing **oligonucleotide** contains one or more phosphorothioate modifications of the phosphate backbone or wherein each phosphate moiety of said phosphate backbone of said **oligonucleotide** is a phosphorothioate modification.

77. The composition of claim 63, wherein said **oligonucleotide** free of unmethylated CpG motifs comprises, or alternatively consists essentially of, or alternatively consists of the sequence GGTCTTTTGGTCCTTGCT.

78. A method of enhancing an immune response against an antigen in an animal comprising introducing into said animal: (a) a virus-like particle bound to at least one antigen capable of inducing an immune response against said antigen in said animal; and (b) at least one

sufficient to enhance the immune response of said animal to said antigen.

79. The method of claim 78, wherein said virus-like particle (a) lacks a lipoprotein-containing envelope.

80. The method of claim 78, wherein said virus-like particle (a) is a recombinant virus-like particle.

81. The method of claim 80, wherein said virus-like particle is selected from the group consisting of: (a) recombinant proteins of Hepatitis B virus; (b) recombinant proteins of measles virus; (c) recombinant proteins of Sindbis virus; (d) recombinant proteins of Rotavirus; (e) recombinant proteins of Foot-and-Mouth-Disease virus; (f) recombinant proteins of Retrovirus; (g) recombinant proteins of Norwalk virus; (h) recombinant proteins of human Papilloma virus; (i) recombinant proteins of BK virus; (o) recombinant proteins of bacteriophages; (k) recombinant proteins of RNA-phages; (l) recombinant proteins of Q β -phage; (m) recombinant proteins of GA-phage; (n) recombinant proteins of fr-phage; (o) recombinant proteins of AP 205-phage; (p) recombinant proteins of Ty; and (q) fragments of any of the recombinant proteins from (a) to (p).

82. The method of claim 81, wherein said virus-like particle is the Hepatitis B virus core protein.

83. The method of claim 78, wherein said antigen (a) is a recombinant antigen.

84. The method of claim 78, wherein said antigen (a) is bound to said virus-like particle by way of a linking sequence.

85. The method of claim 84, wherein said linking sequence comprises a sequence recognized by the proteasome, endosomal proteases or a protease contained in any other vesicular compartment of said antigen presenting cells.

86. The method of claim 84, wherein said virus-like particle is the Hepatitis B virus core protein.

87. The method of claim 78, wherein said antigen (a) is a cytotoxic T cell epitope, a Th cell epitope or a combination of at least two of said epitopes, wherein said at least two epitopes are linked directly or by way of a linking sequence.

88. The method of claim 87, wherein said cytotoxic T cell epitope is a viral or a tumor cytotoxic T cell epitope.

89. The method of claim 87, wherein said antigen is bound to said virus-like particle by way of a linking sequence

90. The method of claim 87, wherein said virus-like particle is the Hepatitis B virus core protein.

91. The method of claim 90, wherein said cytotoxic T cell epitope is fused to the C-terminus of said Hepatitis B virus core protein.

92. The method of claim 91, wherein said cytotoxic T cell epitope is fused to the C-terminus of said Hepatitis B virus core protein by way of a linking sequence.

93. The method of claim 78, wherein said virus-like particle (a) bound to said antigen has the amino acid sequence shown in FIG. 1.

94. The method of claim 78, wherein said antigen (a) is selected from the group consisting of: (a) polypeptides; (b) carbohydrates; (c) steroid hormones; and (d) organic molecules.

95. The method of claim 94, wherein said antigen is an organic molecule.

96. The method of claim 95, wherein said organic molecule is selected from the group consisting of: (a) codeine; (b) fentanyl; (c) heroin; (d) morphium; (e) amphetamine; (f) cocaine; (g) methylenedioxymethamphetamine; (h) methamphetamine; (i) methylphenidate; (j) nicotine; (k) LSD; (l) mescaline; (m) psilocybin; and (n) tetrahydrocannabinol.

97. The method of claim 78, wherein said antigen (a) is derived from the group consisting of: (a) viruses; (b) bacteria; (c) parasites; (d) prions; (e) tumors; (f) self-molecules; (g) non-peptidic haptens; and (h) allergens.

99. The method of claim 98, wherein said tumor antigen is selected from the group consisting of: (a) Her2; (b) GD2; (c) EGF-R; (d) CEA; (e) CD52; (f) human melanoma protein gp100; (g) human melanoma protein melan-A/MART-1; (h) tyrosinase; (i) NA17-A nt protein; (j) MAGE-3 protein; (k) p53 protein; (l) CD21; (m) HPV16 E7 protein; and (n) antigenic fragments of any of the tumor antigens from (a) to (m).

100. The method of claim 78, wherein said virus-like particle comprises recombinant proteins, or fragments thereof, of a RNA-phage.

101. The method of claim 100, wherein said RNA-phage is selected from the group consisting of: (a) bacteriophage Q β ; (b) bacteriophage R17; (c) bacteriophage fr; (d) bacteriophage GA; (e) bacteriophage SP; (f) bacteriophage MS2; (g) bacteriophage M11; (h) bacteriophage MX1; (i) bacteriophage NL95; (k) bacteriophage f2; (l) bacteriophage PP7; and (m) bacteriophage AP205.

102. The method of claim 78, wherein said virus-like particle comprises recombinant proteins, or fragments thereof, of RNA-phage Q β .

103. The method of claim 78, wherein said virus-like particle comprises recombinant proteins, or fragments thereof, of RNA-phage AP 205.

104. The method of claim 78, wherein said substance (b) stimulates upregulation of costimulatory molecules on antigen presenting cells or secretion of cytokines.

105. The method of claim 78, wherein said substance (b) induces nuclear translocation of NF-KB in antigen presenting cells.

106. The method of claim 78, wherein said substance (b) activates toll-like receptors in antigen presenting cells.

107. The method of claim 106, wherein said toll-like receptor activating substance is selected from the group consisting of, or alternatively consists essentially of: (a) immunostimulatory nucleic acids; (b) peptidoglycans; (c) lipopolysaccharides; (d) lipoteichonic acids; (e) imidazoquinoline compounds; (f) flagellines; (g) lipoproteins; (h) immunostimulatory organic molecules; (i) unmethylated CpG-containing **oligonucleotides**; and (j) any mixtures of at least one substance of (a), (b), (c), (d), (e), (f), (g), (h) and/or (i).

108. The method of claim 107, wherein said immunostimulatory nucleic acid is selected from the group consisting of, or alternatively consists essentially of: (a) ribonucleic acids; (b) deoxyribonucleic acids; (c) chimeric nucleic acids; and (d) any mixtures of at least one nucleic acid of (a), (b) and/or (c).

109. The method of claim 108, wherein said ribonucleic acid is poly-(I:C) or a derivative thereof.

110. The method of claim 108, wherein said deoxyribonucleic acid is selected from the group consisting of, or alternatively consists essentially of: (a) unmethylated CpG-containing **oligonucleotides**; and (b) **oligonucleotides** free of unmethylated CpG motifs.

111. The method of claim 78, wherein said immunostimulatory substance is an unmethylated CpG-containing **oligonucleotide**.

112. The method of claim 78, wherein said substance (b) is selected from the group consisting of an anti-CD40 antibody, an immunostimulatory nucleic acid, an unmethylated CpG-containing **oligonucleotide** capable of activating APCs, and a palindromic **oligonucleotide**.

113. The method of claim 78, wherein said unmethylated CpG-containing **oligonucleotide** comprises the sequence: 5'X₁X₂C₆X₃.sub .43' wherein X₁, X₂, X₃, and X₄ are any nucleotide.

114. The method of claim 104, wherein said substance (b) is selected from the group consisting of an anti-CD40 antibody, an immunostimulatory nucleic acid, an unmethylated CpG-containing **oligonucleotide** capable of activating APCs, and a palindromic **oligonucleotide**.

115. The method of claim 105, wherein said substance (b) is selected from the group consisting of an anti-CD40 antibody, an immunostimulatory nucleic acid, an unmethylated CpG-containing **oligonucleotide** capable of activating APCs, and a palindromic **oligonucleotide**.

116. The method of claim 106, wherein said substance (b) is selected from the group consisting of an anti-CD40 antibody, an immunostimulatory nucleic acid, an unmethylated CpG-containing **oligonucleotide** capable

117. The method of claim 113, wherein at least one of said nucleotides X₁, X₂, X₃, and X₄ has a phosphate backbone modification.

118. The method of claim 111, wherein said unmethylated CpG-containing **oligonucleotide** comprises, or alternatively consists essentially of, or alternatively consists of the sequence selected from the group consisting of:

- (a) TCCATGACGTTCTGAATAAT;
- (b) TCCATGACGTTCTGACGTT;
- (c) GGGGTCAACGTTGAGGGGG;
- (d) ATTATTCAGGAACGTCATGGA;
- (e) GGGGGGGGGGACGATCGTCGGGGGGGGG;
- (f) TCCATGACGTTCTGAATAATAAATGCATGTCAAA
GACAGCAT;
- (g) TCCATGACGTTCTGAATAATTCATGACGTT
CCTGAATAATTCATGACGTTCTGAATAAT;
- (h) TCCATGACGTTCTGAATAATCGCGCGCGCGC
GCGC GCGCGCGCGCGCGCGCGCGCGCG; and
- (i) TCGTCGTTTTGTCGTTTTGTCGT.

119. The method of claim 118, wherein said unmethylated CpG-containing **oligonucleotide** contains one or more phosphorothioate modifications of the phosphate backbone or wherein each phosphate moiety of said phosphate backbone of said **oligonucleotide** is a phosphorothioate modification.

120. The method of claim 111, wherein said unmethylated CpG-containing **oligonucleotide** is palindromic.

121. The composition of claim 120, wherein said palindromic unmethylated CpG-containing **oligonucleotide** comprises, or alternatively consists essentially of, or alternatively consists of the sequence GGGGTCAACGTTGAGGGGG.

122. The composition of claim 121, wherein said palindromic unmethylated CpG-containing **oligonucleotide** contains one or more phosphorothioate modifications of the phosphate backbone or wherein each phosphate moiety of said phosphate backbone of said **oligonucleotide** is a phosphorothioate modification.

123. The composition of claim 110, wherein said **oligonucleotide** free of unmethylated CpG motifs comprises, or alternatively consists essentially of, or alternatively consists of the sequence GGTCTTTTGGTCCTTGCT.

124. The method of claim 78, wherein said antigen presenting cell is a dendritic cell, a NK cell, macrophage or a B cell.

125. The method of claim 78, wherein said animal is a mammal.

126. The method of claim 125, wherein said mammal is a human.

127. The method of claim 78, wherein said virus-like particle bound to an antigen (a) and said substance that activates antigen presenting cells (b) are introduced into said animal simultaneously.

128. The method of claim 78, wherein said virus-like particle bound to an antigen (a) and said substance that activates antigen presenting cells (b) are introduced into said animal subcutaneously, intramuscularly or intravenously.

129. The method of claim 78, wherein said immune response is a T cell response and wherein said T cell response against said antigen is enhanced.

130. The method of claim 129, wherein said T cell response is a cytotoxic T cell response and wherein said cytotoxic T cell response against said antigen is enhanced.

131. The method of claim 78, wherein said at least one antigen or antigenic determinant is bound to said virus-like particle by at least one covalent bond, and wherein said covalent bond is a non-peptide bond.

132. The method of claim 78, wherein said at least one antigen or antigenic determinant is fused to said virus-like particle.

133. The method of claim 78, wherein said antigen or antigenic determinant further comprises at least one second attachment site selected from the group consisting of: (a) an attachment site not naturally occurring with said antigen or antigenic determinant; and (b) an attachment site naturally occurring with said antigen or antigenic determinant.

134. The method of claim 78, wherein said composition further comprises an amino acid linker, wherein said amino acid linker comprises, or alternatively consists of, a second attachment site.

135. A method of enhancing an immune response against a virus-like particle in an animal comprising introducing into said animal: (a) a virus-like particle capable of being recognized by the immune system of said animal and inducing an immune response against said virus-like particle in said animal; and (b) at least one substance that activates antigen presenting cells in an amount sufficient to enhance the immune response of said animal to said virus-like particle.

136. The method of claim 135, wherein said virus-like particle (a) lacks a lipoprotein-containing envelope.

137. The method of claim 135, wherein said virus-like particle (a) is a recombinant virus-like particle.

138. The method of claim 137, wherein said virus-like particle is selected from the group consisting of: (a) recombinant proteins of Hepatitis B virus; (b) recombinant proteins of measles virus; (c) recombinant proteins of Sindbis virus; (d) recombinant proteins of Rotavirus; (e) recombinant proteins of Foot-and-Mouth-Disease virus; (f) recombinant proteins of Retrovirus; (g) recombinant proteins of Norwalk virus; (h) recombinant proteins of human Papilloma virus; (i) recombinant proteins of BK virus; (j) recombinant proteins of bacteriophages; (k) recombinant proteins of RNA-phages; (l) recombinant proteins of Q β -phage; (m) recombinant proteins of GA-phage; (n) recombinant proteins of fr-phage; (o) recombinant proteins of AP 205-phage; (p) recombinant proteins of Ty; and (q) fragments of any of the recombinant proteins from (a) to (p).

139. The method of claim 138, wherein said virus-like particle is the Hepatitis B virus core protein.

140. The method of claim 135, wherein said substance (b) stimulates upregulation of costimulatory molecules on antigen presenting cells.

141. The method of claim 135, wherein said substance (b) induces nuclear translocation of NF-KB in antigen presenting cells.

142. The method of claim 135, wherein said substance (b) activates toll-like receptors in antigen presenting cells.

143. The method of claim 142, wherein said toll-like receptor activating substance is selected from the group consisting of, or alternatively consists essentially of: (a) immunostimulatory nucleic acids; (b) peptidoglycans; (c) lipopolysaccharides; (d) lipoteichoic acids; (e) imidazoquinoline compounds; (f) flagellins; (g) lipoproteins; (h) immunostimulatory organic molecules; (i) unmethylated CpG-containing **oligonucleotides**; and (j) any mixtures of at least one substance of (a), (b), (c), (d), (e), (f), (g), (h) and/or (i).

144. The method of claim 143, wherein said immunostimulatory nucleic acid is selected from the group consisting of, or alternatively consists essentially of: (a) ribonucleic acids; (b) deoxyribonucleic acids; (c) chimeric nucleic acids; and (d) any mixtures of at least one nucleic acid of (a), (b) and/or (c).

145. The method of claim 144, wherein said ribonucleic acid is poly-(I:C) or a derivative thereof.

146. The method of claim 144, wherein said deoxyribonucleic acid is selected from the group consisting of, or alternatively consists essentially of: (a) unmethylated CpG-containing **oligonucleotides**; and (b) **oligonucleotides** free of unmethylated CpG motifs.

147. The composition of claim 135, wherein said immunostimulatory

148. The method of claim 135, wherein said substance (b) is selected from the group consisting of an anti-CD40 antibody, an immunostimulatory nucleic acid, an unmethylated CpG-containing **oligonucleotide** capable of activating APCs, and a palindromic **oligonucleotide**.

149. The method of claim 147, wherein said unmethylated CpG-containing **oligonucleotide** comprises the sequence: 5'X₁X₂CX₃X₄.sub .43' wherein X₁, X₂, X₃, and X₄ are any nucleotide.

150. The method of claim 140, wherein said substance (b) is selected from the group consisting of an anti-CD40 antibody, an immunostimulatory nucleic acid, an unmethylated CpG-containing **oligonucleotide** capable of activating APCs, and a palindromic **oligonucleotide**.

151. The method of claim 141, wherein said substance (b) is selected from the group consisting of an anti-CD40 antibody, an immunostimulatory nucleic acid, an unmethylated CpG-containing **oligonucleotide** capable of activating APCs, and a palindromic **oligonucleotide**.

152. The method of claim 142, wherein said substance (b) is selected from the group consisting of an anti-CD40 antibody, an immunostimulatory nucleic acid, an unmethylated CpG-containing **oligonucleotide** capable of activating APCs, and a palindromic **oligonucleotide**.

153. The method of claim 135, wherein said antigen presenting cell is a dendritic cell, a NK cell, macrophage or a B cell.

154. The method of claim 135, wherein said animal is a mammal.

155. The method of claim 154, wherein said mammal is a human.

156. The method of claim 135, wherein said virus-like particle (a) and said substance that activates antigen presenting cells (b) are introduced into said animal simultaneously.

157. The method of claim 135, wherein said virus-like particle (a) and said substance that activates antigen presenting cells (b) are introduced into said animal subcutaneously, intramuscularly or intravenously.

158. The method of claim 135, wherein said immune response is a T cell response and wherein said T cell response against said antigen is enhanced.

159. The method of claim 158, wherein said T cell response is a cytotoxic T cell response and wherein said cytotoxic T cell response against said antigen is enhanced.

160. The method of claim 149, wherein at least one of said nucleotides X₁, X₂, X₃, and X₄ has a phosphate backbone modification.

161. The method of claim 147, wherein said unmethylated CpG-containing **oligonucleotide** comprises, or alternatively consists essentially of, or alternatively consists of the sequence selected from the group consisting of:

- (a) TCCATGACGTTCTGAATAAT;
- (b) TCCATGACGTTCTGACGTT;
- (c) GGGGTCAACGTTGAGGGGG;
- (d) ATTATTCAGGAACGTCATGGA;
- (e) GGGGGGGGGGACGATCGTCGGGGGGGGG;
- (f) TCCATGACGTTCTGAATAATAATGCATGTCAA
GACAGCAT;
- (g) TCCATGACGTTCTGAATAATTCCATGACGTT
CCTGAATAATTCCATGACGTTCTGAATAAT;
- (h) TCCATGACGTTCTGAATAATCGCGCGCGCGC
GCGC GCGCGCGCGCGCGCGCGCGCGCG; and
- (i) TCGTCGTTTTGTCGTTTTGTCGT.

oligonucleotide contains one or more phosphorothioate modifications of the phosphate backbone or wherein each phosphate moiety of said phosphate backbone of said **oligonucleotide** is a phosphorothioate modification.

163. The composition of claim 147, wherein said unmethylated CpG-containing **oligonucleotide** is palindromic.

164. The composition of claim 163, wherein said palindromic unmethylated CpG-containing **oligonucleotide** comprises, or alternatively consists essentially of, or alternatively consists of the sequence
GGGGTCAACGTTGAGGGGG.

165. The composition of claim 164, wherein said palindromic unmethylated CpG-containing **oligonucleotide** contains one or more phosphorothioate modifications of the phosphate backbone or wherein each phosphate moiety of said phosphate backbone of said **oligonucleotide** is a phosphorothioate modification.

166. The composition of claim 146, wherein said **oligonucleotide** free of unmethylated CpG motifs comprises, or alternatively consists essentially of, or alternatively consists of the sequence
GGTTCTTTGGTCCTTGCTCT.

167. A vaccine comprising an immunologically effective amount of the composition of claim 1 together with a pharmaceutically acceptable diluent, carrier or excipient.

168. The vaccine of claim 167 further comprising an adjuvant.

169. A vaccine comprising an immunologically effective amount of the composition of claim 52 together with a pharmaceutically acceptable diluent, carrier or excipient.

170. The vaccine of claim 169 further comprising an adjuvant.

171. A method of immunizing or treating an animal comprising administering to said animal an immunologically effective amount of the vaccine of claim 167.

172. The method of claim 171, wherein said animal is a mammal.

173. The method of claim 172, wherein said animal is a human.

174. A method of immunizing or treating an animal comprising administering to said animal an immunologically effective amount of the vaccine of claim 169.

175. The method of claim 174, wherein said animal is a mammal.

176. The method of claim 175, wherein said animal is a human.

177. A method of enhancing anti-viral protection in an animal comprising introducing into said animal the composition of claim 1.

178. A method of enhancing anti-viral protection in an animal comprising introducing into said animal the composition of claim 52.

179. A method of immunizing or treating an animal comprising priming a T cell response in said animal by administering an immunologically effective amount of the vaccine of claim 167.

180. The method of claim 179 further comprising the step of boosting the immune response in said animal.

181. The method of claim 180, wherein said boosting is effected by administering an immunologically effective amount of a vaccine of claim 168 or an immunologically effective amount of a heterologous vaccine.

182. The method of claim 181, wherein said heterologous vaccine is a DNA vaccine or a viral vaccine or a canary pox vaccine.

183. A method of immunizing or treating an animal comprising boosting a T cell response in said animal by administering an immunologically effective amount of the vaccine of claim 167.

184. The method of claim 183 further comprising the step of priming a T cell response in said animal.

185. The method of claim 184, wherein said priming is effected by administering an immunologically effective amount of a vaccine of claim 168 or an immunologically effective amount of a heterologous vaccine.

186. The method of claim 185, wherein said heterologous vaccine is a DNA vaccine or a viral vaccine or a canary pox vaccine.

187. A method of immunizing or treating an animal comprising priming a T cell response in said animal by administering an immunologically effective amount of the vaccine of claim 169.

188. The method of claim 187 further comprising the step of boosting the immune response in said animal.

189. The method of claim 188, wherein said boosting is effected by administering an immunologically effective amount of a vaccine of claim 170 or an immunologically effective amount of a heterologous vaccine.

190. The method of claim 189, wherein said heterologous vaccine is a DNA vaccine or a viral vaccine or a canary pox vaccine.

191. A method of immunizing or treating an animal comprising boosting a T cell response in said animal by administering an immunologically effective amount of the vaccine of claim 169.

192. The method of claim 191 further comprising the step of priming a T cell response in said animal.

193. The method of claim 192, wherein said priming is effected by administering an immunologically effective amount of a vaccine of claim 170 or an immunologically effective amount of a heterologous vaccine.

194. The method of claim 193, wherein said heterologous vaccine is a DNA vaccine or a viral vaccine or a canary pox vaccine.

L5 ANSWER 58 OF 112 USPTAFULL on STN

2003:120747 Blood cell deficiency treatment method.

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DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention relates to the use of compounds to treat a number of conditions, such as thrombocytopenia, neutropenia or the delayed effects of radiation therapy. Compounds that can be used in the invention include methyl-2,3,4-trihydroxy-1-O-(7,17-dioxoandrost-5-ene-3 β -yl)- β -D-glucopyranosiduronate, 16 α ,3 α -dihydroxy-5 α -androstan-17-one or 3,7,16,17-tetrahydroxyandrost-5-ene, 3,7,16,17-tetrahydroxyandrost-4-ene, 3,7,16,17-tetrahydroxyandrost-1-ene or 3,7,16,17-tetrahydroxyandrostane that can be used in the treatment method.

CLM What is claimed is:

1. A method to treat a blood cell deficiency in a subject in need thereof comprising administering to the subject, or delivering to the subject's tissues, an effective amount of a compound of formula 1 ##STR115## wherein, each R¹, R², R³, R⁴, R⁵, R⁶ and R¹⁰ independently are --H, OH, --OR^{PR}, --SR^{PR}, --N(R^{PR})₂, --O--Si--(R¹³)₃, --CHO, --CHS, --CH.dbd.NH, --CN, --SCN, --NO₂, --OSO_{3H}, --OPO_{3H}, an ester, a thioester, a thionoester, a phosphoester, a phosphothioester, a phosphonoester, a phosphiniester, a sulfite ester, a sulfate ester, an amide, an amino acid, a peptide, an ether, a

halogen, an optionally substituted alkyl group, an optionally substituted alkenyl group, an optionally substituted alkynyl group, an optionally substituted aryl moiety, an optionally substituted heteroaryl moiety, an optionally substituted heterocycle, an optionally substituted monosaccharide, an optionally substituted oligosaccharide, a nucleoside, a nucleotide, an **oligonucleotide**, a polymer, or, one or more of both R¹, R², R³ or R⁴ together comprise an independently selected spiro ring, or one more of R¹, R², R³, R⁴, R⁵, R⁶ and R¹⁰ are .dbd.O, .dbd.S, .dbd.N--OH, .dbd.CH₂, or a spiro ring and the hydrogen atom or the second variable group that is bonded to the same carbon atom is absent, or, one or more of two adjacent R¹--R⁶ and R¹⁰ comprise an independently selected an acetal, a thioacetal, ketal or thioketal, or all R³ and R⁴ together comprise a structure of formula 2

##STR116## R¹ is --C(R¹⁰)₂--, --C(R¹⁰)₂--C(R¹⁰)₂--, --C(R¹⁰)₂--C(R¹⁰)₂--C(R¹⁰)₂--, --C(R¹⁰)₂--O--C(R¹⁰)₂--, --C(R¹⁰)₂--S--C(R¹⁰)₂--, --C(R¹⁰)₂--NRPR--C(R¹⁰)₂--, --O--C(R¹⁰)₂--, --S--C(R¹⁰)₂--, --NRPR-- or --NRPR--C(R¹⁰)₂--; R⁸ and R⁹ independently are --C(R¹⁰)₂--, --C(R¹⁰)₂--C(R¹⁰)₂--, --O--C(R¹⁰)₂--, --S--C(R¹⁰)₂--, --NRPR--C(R¹⁰)₂--, or one or both of R⁸ or R⁹ independently are absent, leaving a 5-membered ring; R¹³ independently is C₁₋₆ alkyl; R^{PR} independently is --H or a protecting group; D is a heterocycle or a 4-, 5-, 6- or 7-membered ring that comprises saturated carbon atoms, wherein 1, 2 or 3 ring carbon atoms of the 4-, 5-, 6- or 7-membered ring are optionally independently substituted with --O--C(R¹⁰)₂--, --S--C(R¹⁰)₂--, --NRPR--C(R¹⁰)₂-- or where 1, 2 or 3 hydrogen atoms of the heterocycle or where 1, 2 or 3 hydrogen atoms of the 4-, 5-, 6- or 7-membered ring are substituted with --OH, --OR^{PR}, --SR^{PR}, --N(R^{PR})₂, --O--Si--(R¹³)₃, --CHO, --CHS, --CH.dbd.NH, --CN, --SCN, --NO₂, --OSO₃H, --OPO₃H, an ester, a thioester, a thionoester, a phosphoester, a phosphothioester, a phosphiniester, a sulfite ester, a sulfate ester, an amide, an amino acid, a peptide, an ether, a thioether, an acyl group, a thioacyl group, a carbonate, a carbamate, a halogen, an optionally substituted alkyl group, an optionally substituted alkenyl group, an optionally substituted alkynyl group, an optionally substituted aryl moiety, an optionally substituted heteroaryl moiety, an optionally substituted heterocycle, an optionally substituted monosaccharide, an optionally substituted oligosaccharide, a nucleoside, a nucleotide, an **oligonucleotide** or a polymer, or, one more of the ring carbons in D are substituted with .dbd.O, .dbd.S, .dbd.N--OH, .dbd.CH₂, or a spiro ring, or one or more of two adjacent ring carbons in D comprise an independently selected acetal, thioacetal, ketal or thioketal, or D comprises two 5- or 6-membered rings, wherein the rings are fused or are linked by 1 or 2 bonds, or a metabolic precursor or a biologically active metabolite thereof, provided that the compound is not 5-androstene-3 β -ol-17-one, 5-androstene-3 β ,17 β -diol, 5-androstene-3 β ,7 β ,17 β -triol or a derivative of any of these three compounds that can convert to these compounds by hydrolysis.

2. The method of claim 1 wherein one or two R¹⁰ at the 1, 4, 6, 8, 9, 12 and 14 positions is not --H.

3. The method of claim 2 wherein the one or two R¹⁰ at the 1, 4, 6, 8, 9, 12 and 14 positions are independently selected from --F, --Cl, --Br, --I, --OH, .dbd.O, --CH₃, --C₂H₅, an ether optionally selected from --OCH₃ and --OC₂H₅, and an ester optionally selected from --O--C(O)--CH₃ and --O--C(O)--C₂H₅.

4. The method of claim 3 wherein the one or two R¹⁰ at the 1, 4, 6, 8, 9, 12 and 14 positions are independently selected from --F and --OH.

5. The method of claim 4 wherein R¹, R², R³ and R⁴ are independently selected from --H, --OH, .dbd.O, an ester and an ether.

6. The method of claim 1 wherein the subject has thrombocytopenia or neutropenia.

7. The method of claim 1 wherein the subject's circulating platelets, red cells, mature myelomonocytic cells, or their precursor cells, in circulation or in tissue is detectably increased.

8. The method of claim 7 wherein the subject's circulating platelets are

9. The method of claim 7 wherein the subject's circulating myelomonocytic cells are detectably increased.
10. The method of claim 7 wherein the circulating myelomonocytic cells are neutrophils.
11. The method of claim 7 wherein the myelomonocytic cells are basophils, neutrophils or eosinophils.
12. The method of claim 7 wherein the subject's circulating red cells are detectably increased.
13. The method of claim 7 wherein the subject is has renal failure.
14. The method of claim 7 further comprising the steps of obtaining blood from the subject before administration of the formula 1 compound and measuring the subject's white or red cell counts and optionally, on one, two, three or more occasions, measuring the subject's circulating white cell or red cell counts after administration of the formula 1 compound.
15. The method of claim 14 wherein the subject's white or red cell counts are measured on one, two, three or more occasions within about 12 weeks after an initial administration of the formula 1 compound.
16. A compound of formula V ##STR117## or a pharmaceutically acceptable salt, ester, amide, or prodrug thereof, wherein (a) R₁ and R₂ are each independently selected from the group consisting of a hydrogen atom and a glucuronide group having the formula ##STR118## wherein (i) R₇ is an alkyl ester wherein the alkyl moiety is optionally substituted, and (ii) R₈, R₉ and R₁₀ are each --OR₁₄, wherein R₁₄ is a hydrogen atom or a protected hydroxy, optionally substituted alkyl, cycloalkyl; and (iii) at least one of R₁ or R₂ is not hydrogen; (b) R₅ and R₆ are each independently selected from the group consisting of a hydrogen atom, optionally substituted alkyl, hydroxy, and a protected hydroxy; or R₅ and R₆ taken together are a ketone group (.dbd.O); and (c) R₁₂ and R₁₃ are each independently selected from the group consisting of a hydrogen atom, optionally substituted alkyl, hydroxy, and a protected hydroxy.
17. The compound of claim 16, wherein said protected hydroxy is an ester or wherein one of R₁ and R₂ is --H and the other one of R₁ and R₂ is the glucuronide group.
18. The compound of claim 16, wherein R₁₂ and R₁₃ are methyl.
19. A composition comprising a compound of claim 16 and one or more excipients.
20. A compound of formula VII ##STR119## or a pharmaceutically acceptable salt, ester, amide, or prodrug thereof, wherein (a) R₃ and R₄ are each independently selected from the group consisting of a hydrogen atom and a glucuronide group having the formula ##STR120## wherein (i) R₇ is an alkyl ester wherein the alkyl moiety is optionally substituted, and (ii) R₈, R₉ and R₁₀ are each --OR₁₄, wherein R₁₄ is a hydrogen atom, optionally substituted alkyl, cycloalkyl, or a protected hydroxy; and (iii) at least one of R₃ and R₄ is not hydrogen; (b) R₅ and R₆ are each independently selected from the group consisting of a hydrogen atom, optionally substituted alkyl, hydroxy, and a protected hydroxy; or R₅ and R₆ taken together are .dbd.O; (c) R₁₁ is a hydrogen atom or a protected hydroxy; and (d) R₁₂ and R₁₃ are each independently selected from the group consisting of a hydrogen atom, optionally substituted alkyl, hydroxy, and a protected hydroxy.
21. The compound of claim 20, wherein one of R₃ and R₄ is a hydrogen atom and the other one of R₁ and R₂ is the glucuronide.
22. The compound of claim 20, wherein one of R₅ and R₆ is a hydrogen atom and the other one of R₅ and R₆ is acetoxy.
23. The compound of claim 20, wherein R₁₂ and R₁₃ are methyl.
24. The compound of claim 20 selected from the group consisting of methyl-2,3,4-tri-O-acetyl-1-O-(3 β ,7 β -diacetoxyandrost-5-ene-7 β -yl)- β -D-glucopyranosiduronate, methyl 1-O-(3 β ,17 β -diacetoxyandrost-5-ene-7 β -yl)- β -D-glucopyranosiduronate, and

7 α -yl)- β -D-glucopyranosiduronate, or the pharmaceutically acceptable salt, ester, ether, amide, or prodrug thereof.

25. A compound of formula IX ##STR121## or a pharmaceutically acceptable salt, ester, amide, or prodrug thereof, wherein (a) R₁ and R₂ are each independently selected from the group consisting of a hydrogen atom and --O--C(O)--OR₁₄, wherein (i) R₁₄ is selected from the group consisting of a hydrogen atom, optionally substituted alkyl, and carbocyclic ring (cycloalkyl); and (ii) at least one of R₁ or R₂ is not hydrogen; (b) R₅, R₆, R₇, and R₈ are each independently selected from the group consisting of a hydrogen atom, optionally substituted alkyl, hydroxy, --O--C(O)--OR₁₄, and a protected hydroxy; or R₅ and R₆ taken together form an oxygen atom, which, together with the carbon atom to which R₅ and R₆ are joined, forms a ketone group; or R₇ and R₈ taken together form an oxygen atom, which, together with the carbon atom to which R₇ and R₈ are joined, forms a ketone group; and (c) R₁₂ and R₁₃ are each independently selected from the group consisting of a hydrogen atom, optionally substituted alkyl, hydroxy, and a protected hydroxy.

26. The compound of claim 25, wherein the protected hydroxy is an ester.

27. The compound of claim 25, wherein one of R₁ and R₂ is a hydrogen atom and the other one of R₁ and R₂ is --O--C(O)--OR₁₄.

28. The compound of claim 27, wherein R₁₄ is selected from the group consisting of methyl, ethyl, n-propyl, i-propyl, n-butyl, sec-butyl, t-butyl, pentyl, hexyl, n-octyl, n-dodecyl, 1-ethoxyethyl, 9-fluorenylmethyl, --CH₂--C(O)CH₃ and --C(O)CH₃.

29. The compound of claim 25, wherein R₅ and R₆ are each independently selected from the group consisting of --H, --OH, --O--C(O)--OCH₃, --O--C(O)--OC₂H₅, --O--C(O)--OC₃H₇, --O--C(O)--OC₄H₉, --O--C(O)--OCH₂C₂H₅, --O--C(O)--OCH₂C₃H₇ and --O--C(O)--O--(CH₂)₂--O--C₂H₅ or together are .dbd.O.

30. The compound of claim 25, wherein R₁₂ and R₁₃ are methyl.

31. The compound of claim 25 selected from the group consisting of 3 β -carbomethoxyandrost-5-ene-7,17-dione, 3 β -carboallyloxyandrost-5-ene-7,17-dione, 3 β -carboethoxyandrost-5-ene-7,17-dione, 3 β -carboisobutoxyandrost-5-ene-7,17-dione, 3 β ,17 β -dicaromethoxyandrost-5-ene-7-one, 3 β -carbooctyloxyandrost-5-ene-7,17-dione, 3 β -carbo(9-fluorenyl)methoxyandrost-5-ene-7,17-dione, 3 β -carbomethoxyandrost-5-ene-7,17 β -diol, 3 β -carboethoxyandrost-5-ene-7 β ,17 β -diol, and 3 β -carbooctyloxyandrost-5-ene-7 β ,17 β -diol, or the pharmaceutically acceptable salt, ester, ether, amide, or prodrug thereof.

32. A composition comprising a compound of claim 25 and one or more excipients.

33. A method to treat a symptom or condition associated with one or more delayed adverse or unwanted effects of radiation exposure in a subject in need thereof comprising administering to the subject, or delivering to the subject's tissues, an effective amount of a compound of formula I ##STR122## wherein, each R₁, R₂, R₃, R₄, R₅, R₆ and R₁₀ independently are --H, --OH, --OR^{PR}, --SR^{PR}, --N(R^{PR})₂, --O--Si--(R¹³)₃, --CHO, --CHS, --CH.dbd.NH, --CN, --SCN, --NO₂, --OSO₃H, --OPO₃H, an ester, a thioester, a thionoester, a phosphoester, a phosphothioester, a phosphonoester, a phosphiniester, a sulfite ester, a sulfate ester, an amide, an amino acid, a peptide, an ether, a thioether, an acyl group, a thioacyl group, a carbonate, a carbamate, a halogen, an optionally substituted alkyl group, an optionally substituted alkenyl group, an optionally substituted alkynyl group, an optionally substituted aryl moiety, an optionally substituted heteroaryl moiety, an optionally substituted heterocycle, an optionally substituted monosaccharide, an optionally substituted oligosaccharide, a nucleoside, a nucleotide, an **oligonucleotide**, a polymer, or, one or more of both R₁, R₂, R₃ or R₄ together comprise an independently selected spiro ring, or one more of R₁, R₂, R₃, R₄, R₅, R₆ and R₁₀ independently are .dbd.O, .dbd.S, .dbd.N--OH, .dbd.CH₂, or a spiro ring, and the hydrogen atom or the

or, one or more of two adjacent R¹--R⁶ and R¹⁰ comprise an independently selected acetal, thioacetal, ketal or thioketal moiety; all R³ and R⁴ together comprise a structure of formula 2

##STR123## R is --C(R¹⁰)₂--, --C(R¹⁰)₂--C(R¹⁰)₂--, --C(R¹⁰)₂--C(R¹⁰)₂--C(R¹⁰)₂--, --C(R¹⁰)₂--O--C(R¹⁰)₂--, --C(R¹⁰)₂--S--C(R¹⁰)₂--, --C(R¹⁰)₂--NRPR--C(R¹⁰)₂--, --O--C(R¹⁰)₂--, --O--C(R¹⁰)₂--S--C(R¹⁰)₂--, --NRPR--C(R¹⁰)₂-- or --NRPR--C(R¹⁰)₂--; R⁸ and R⁹ independently are --C(R¹⁰)₂--, --C(R¹⁰)₂--C(R¹⁰)₂--, --O--C(R¹⁰)₂--, --S--C(R¹⁰)₂--, --NRPR--C(R¹⁰)₂-- or --NRPR--C(R¹⁰)₂--, or one or both of R⁸ or R⁹ independently are absent, leaving a 5-membered ring; R¹³ independently is C₁₋₆ alkyl; R^{PR} independently is --H or a protecting group; D is a heterocycle or a 4-, 5-, 6- or 7-membered ring that comprises saturated carbon atoms, wherein 1, 2 or 3 ring carbon atoms of the 4-, 5-, 6- or 7-membered ring are optionally independently substituted with --O--C(R¹⁰)₂--, --S--C(R¹⁰)₂-- or --NRPR--C(R¹⁰)₂-- or where 1, 2 or 3 hydrogen atoms of the heterocycle or where 1, 2 or 3 hydrogen atoms of the 4-, 5-, 6- or 7-membered ring are independently substituted with --OH, --OR^{PR}, --SR^{PR}, --N(R^{PR})₂, --O--Si--(R¹³)₃, --CHO, --CH₂--, --CH₂--dbd.NH, --CN, --SCN, --NO₂, --OSO₃H, --OPO₃H, an ester, a thioester, a thionoester, a phosphoester, a phosphothioester, a phosphiniester, a sulfite ester, a sulfate ester, an amide, an amino acid, a peptide, an ether, a thioether, an acyl group, a thioacyl group, a carbonate, a carbamate, a halogen, an optionally substituted alkyl group, an optionally substituted alkenyl group, an optionally substituted alkynyl group, an optionally substituted aryl moiety, an optionally substituted heteroaryl moiety, an optionally substituted heterocycle, an optionally substituted monosaccharide, an optionally substituted oligosaccharide, a nucleoside, a nucleotide, an **oligonucleotide** or a polymer, or, one more of the ring carbons are substituted with .dbd.O, .dbd.S, .dbd.N--OH, .dbd.CH₂, or a spiro ring, or two adjacent D ring carbons comprise an independently selected acetal, thioacetal, ketal or thioketal moiety, or D comprises two 5- or 6-membered rings, wherein the rings are fused or are linked by 1 or 2 bonds, and the dotted lines are optional double bonds, provided that there are not double bonds simultaneously at the 4-5 and the 5-6 positions. wherein the formula 1 compound is administered or delivered to the subject's tissues beginning at least 1 day after the subject has been exposed to a dose of radiation that will cause or could potentially cause the one or more delayed adverse or unwanted effects of the radiation exposure or wherein the formula 1 compound is administered or delivered to the subject's tissues beginning at least 1 day after the subject has been exposed to at least one subdose of a planned course of radiation exposures that will cause or could potentially cause the one or more delayed adverse effects or unwanted effects of the radiation exposure.

34. The method of claim 33 wherein the subject has received a total radiation dose of at least about 0.5 Gy to about 300 Gy, at least about Gy 1 to about 200 Gy or at least about Gy 2 to about 150 Gy, wherein the subject received the radiation dose in a single dose or in two or more divided doses.

35. The method of claim 33 wherein the symptom or condition associated with one or more delayed adverse effect of radiation is one or more of encephalopathy, myelopathy, nausea, vomiting, diarrhea, acute inflammation, chronic inflammation, edema, pain, headache, depression, fever, malaise, weakness, hair loss, skin atrophy, skin ulceration, skin lesion, keratosis, telangiectasia, infection, hypoplasia, atrophy, fibrosis, pneumonitis, bone marrow hypoplasia, hemorrhage or cytopenia.

36. The method of claim 35 wherein the infection is a bacterial, viral, fungal, parasite or yeast infection, or wherein the fibrosis is lung fibrosis or wherein the cytopenia is anemia, leukopenia or thrombocytopenia.

37. The method of claim 33 wherein the symptom or condition associated with one or more delayed adverse or unwanted effect of the radiation exposure is caused by or associated with radiation damage to one or more of bone marrow cells, bowel epithelium, bone marrow, testicles, ovaries, brain nerves or tissue, peripheral nerves, spinal cord nerves or tissue or skin epithelium.

38. The method of claim 33 wherein the subject has received or will receive a total radiation dose of at least about 0.5 Gy, at least about 2 Gy, at least about 4 Gy or at least about 6 Gy.

39. The method of claim 33 wherein the subject has received or is

e.g., about 10, 20, 30, 40, 50, 100, 150, 200 or 300 Gy.

40. The method of claim 33 wherein about 0.1 mg/kg/day to about 50 mg/kg/day of the formula 1 compound is administered to the subject or delivered to the subject's tissues.

41. The method of claim 33 wherein the formula 1 compound has the structure B ##STR124## wherein R¹ is --H, --OH, .dbd.O, --SH, .dbd.S, --OCH₃, --OC₂H₅, --O--S(O)(O)--O-Na⁺, --O--S(O)(O)--OC₂H₅, --CH₃, --C₂H₅, --OC(O)C(CH₃)₃, --OC(O)CH₃, an optionally substituted monosaccharide, an optionally substituted oligosaccharide comprising two, three or more covalently linked optionally substituted monosaccharides, or an amino acid; R² is --H, --OH, .dbd.O, --CH₃, --CF₃, --OCH₃, --OC₂H₅, --C₂H₅, --OCH₂CH₂CH₃, --OCH₂CH₂CH₂CH₃, --F, --Cl, --Br or --I; R³ is --H, --F, --Cl, --Br, --I, --OH, --SH, .dbd.O, .dbd.CH₂, --NH₂, --CH₃, --CF₃, --C₂H₅, --O--C(O)--CH₃, --O--C(O)--CH₂CH₃, --O--C(O)--CH₂CH₂CH₃, --C(O)--CH₃, --C(O)--CH₂CH₃, --C(O)--CH₂CH₂CH₃; R⁴ is --H, --F, --Cl, --Br, --I, --OH, .dbd.O, .dbd.CH₂, --CCH, --SH --O--C(O)--CH₃, --O--C(O)--CH₂CH₃, --O--C(O)--CH₂CH₂CH₃, --C(O)--CH₃, --C(O)--CH₂CH₃, --C(O)--CH₂CH₂CH₃, --CHOH--CH₃, --CHOH--CH₂CH₃, --CHOH--CH₂CH₂CH₃, --CHOH--C₆H₁₃, an optionally substituted monosaccharide, an optionally substituted oligosaccharide comprising two, three or more covalently linked optionally substituted monosaccharides or an amino acid; R⁵ and R⁶ are independently --H, --CH₃, --CH₂OH, --CHO, --CH₂F, --CH₂Cl, --CH₂Br, --CH₂I; R⁷ is --CH₂--, --CHF--, --CHCl--, --CHBr--, --CHI--, --C(CH₃)-- or --CH(C₁₋₈ alkyl, e.g., --CH(CH₃)--, --CH(C₂H₅)-- or --CH(C₃H₇)--); R⁸ is --CH₂--, --CHF--, --CHCl--, --CHBr--, --CHI--, --C(CH₃)--, --CH(CH₃)--, --CH(C₂H₅)-- or --CH(C₃H₇)--; R⁹ is --CH₂--, --CHOH--, --CHF--, --CHCl--, --CHBr--, --CHI--, --C(CH₃)--, --CH(CH₃)--, --CH(C₂H₅)--, --CH(C₃H₇)--, --CH(OCH₃)--, --CH(OC₂H₅)-- or --CH(OC₃H₇)--; and the hydrogen atom at the 5-position, if present, is in the α- or β-configuration.

42. The method of claim 41 wherein R¹, if monovalent, is in the β-configuration.

43. The method of claim 41 wherein R¹, if monovalent, is in the α-configuration.

44. The method of claim 41 wherein R⁷, R⁸ and R⁹ independently are --CH₂--, --CHF--, --CHCl--, --CHBr--, --CHI--, --CH(C₁₋₈ alkyl)- or --CHOH--.

45. The method claim 41 wherein the formula 1 compound is 16α-bromoepiandrosterone, 16α-bromoepiandrosterone hemihydrate, 16α-hydroxyepiandrosterone, 3α,16α-dihydroxy-5α-androstane-17-one, 3α,16α,17β-trihydroxy-5α-androstane, 3α,16α,17α-trihydroxy-5α-androstane, 3β,17β-dihydroxyandrost-5-ene or 3β,7β,17β-trihydroxyandrost-5-ene, 7-oxodehydroepiandrosterone, 16α-fluoroandrost-5-ene-17-one, 7α-hydroxy-16α-fluoroandrost-5-ene-17-one, 7β-hydroxy-16α-fluoroandrost-5-ene-17-one, 3α-hydroxy-16α-fluoroandrost-5-ene-17-one, 3β-hydroxy-16α-fluoroandrost-5-ene-17-one, 3β,7β-dihydroxy-16α-fluoroandrost-5-ene-17-one, 3α,7α-dihydroxy-16α-fluoroandrost-5-ene-17-one, 3α,7β-dihydroxy-16α-fluoroandrost-5-ene-17-one, 3α,7α-dihydroxy-16α-fluoroandrost-5-ene-17-one, 7β,17β-dihydroxy-16α-fluoroandrost-5-ene, 7α,17β-dihydroxy-16α-fluoroandrost-5-ene, 7β,17α-dihydroxy-16α-fluoroandrost-5-ene, 7α,17α-dihydroxy-16α-fluoroandrost-5-ene, 3β,17β-dihydroxy-16α-fluoroandrost-5-ene, 3α,17β-dihydroxy-16α-fluoroandrost-5-ene, 3β,17α-dihydroxy-16α-fluoroandrost-5-ene,

17 α -hydroxy-16 α -fluoroandroster-5-ene, 17 β -hydroxy-16 α -fluoroandroster-5-ene or an ester, ether, sulfate or glucuronide of any of these compounds having a hydroxyl moiety.

L5 ANSWER 59 OF 112 USPTAFULL on STN

2003:120098 Protein arrays and methods and systems for producing the same.

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US 2003082579 A1 20030501

APPLICATION: US 2002-159428 A1 20020529 (10)

PRIORITY: US 2001-294739P 20010530 (60)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Methods of rapidly generating and analyzing a plurality of polypeptides are disclosed. More specifically, libraries and arrays of polypeptides are assayed in order to determine their individual immunogenic effect. Based on the immunogenic effect of polypeptides, specific subunit vaccines can be developed.

CLM What is claimed is:

1. A method of generating a library of target organism polypeptides, comprising the following steps: (a) performing a first **PCR** reaction using a first **primer** pair capable of amplifying a desired polynucleotide sequence from the target organism to provide an amplified coding sequence, which amplified coding sequence is not transcriptionally active; (b) providing a second **PCR** nucleotide **primer** pair capable of adding at least one nucleotide sequence that confers transcriptional activity to the amplified coding sequence; (c) performing a second **PCR** reaction with the second **primer** pair and the amplified coding sequence, thereby resulting in amplification of a transcriptionally active coding sequence; (d) expressing the polypeptide of the transcriptionally active coding sequence; and (e) repeating steps (a)-(d) at least 10 times, with different first **primer** pairs to express different polypeptides of said target organism.

2. The method of claim 1, wherein steps (a)-(d) are repeated at least 20 times.

3. The method of claim 1, wherein steps (a)-(d) are repeated at least 100 times.

4. The method of claim 1, wherein steps (a)-(d) are repeated about 266 times.

5. The method of claim 1, wherein the target organism is Vaccinia virus.

6. The method of claim 1, further comprising adding at least one polynucleotide sequence operably encoding a linker molecule to the amplified coding sequence or the transcriptionally active coding sequence, wherein the linker molecule immobilizes the polypeptide to a solid support.

7. The method of claim 6, wherein expressing the transcriptionally active coding sequence and the polynucleotide sequence operably encoding a linker molecule produces a target organism polypeptide attached to a linker molecule.

8. The method of claim 7, wherein the linker molecule is an epitope.

9. The method of claim 7, wherein the linker molecule is selected from the group consisting of 6x, 7x, 8x, 9x, and 10x his-tag, GST tag, fluorescent protein tag, Flag tag, and HA tag

10. The method of claim 1, wherein the at least one sequence that confers transcriptional activity is a promoter sequence.

11. The method of claim 1, wherein the at least one sequence that confers transcriptional activity is a terminator sequence.

12. The method of claim 1, further comprising designing the first **primer** pair using an automated system.

13. The method of claim 1, wherein the first **PCR** reaction is performed using an automated system.

14. The method of claim 1, wherein the second **PCR** reaction is performed using an automated system.

15. The method of claim 1, wherein the transcriptionally active coding sequence is expressed using an automated system.

order to identify a target organism antigen that is capable of eliciting a humoral immune response, comprising: providing a library of target organism polypeptides attached to a linker molecule prepared according to the method of claim 7; immobilizing at least 10 of the target organism polypeptides to a solid support; and assaying the polypeptides with at least one antibody from an animal that has been immunized with one or more antigens from the target organism to identify a target organism antigen capable of eliciting a humoral immune response.

17. The method of claim 16, wherein the target organism is Vaccinia virus.

18. A method of screening a library of target organism polypeptides in order to identify a target organism antigen that is capable of eliciting a cell-mediated immune response, comprising: providing a library of target organism polypeptides according to the method of claim 1; delivering at least 10 of the target organism polypeptides into a plurality of antigen-presenting cells; and assaying the antigen-presenting cells with at least one T-cell from an animal that has been immunized with one or more antigens from the target organism to identify a target organism antigen capable of eliciting a cell-mediated immune response.

19. The method of claim 18, wherein the target organism is Vaccinia virus.

20. The method of claim 18, wherein the antigen-presenting cells are B cells.

21. The method of claim 18, wherein the antigen-presenting cells are macrophages.

22. The method of claim 18, wherein the antigen-presenting cells are dendritic cells.

23. A method of developing a subunit vaccine against a target organism, comprising: providing a target organism antigen that is capable of eliciting an immune response, identified according to the method of claim 16; administering the antigen alone or in combination with at least one other target organism antigen that is capable of eliciting an immune response to a subject; and monitoring the generation of an immune response to the antigen or combination of the antigens in the subject.

24. The method of claim 23, wherein the target organism is Vaccinia virus.

25. A method of developing a subunit vaccine against a target organism, comprising: providing a target organism antigen that is capable of eliciting an immune response, identified according to the method of claim 18; administering the antigen alone or in combination with at least one other target organism antigen that is capable of eliciting an immune response to a subject; and monitoring the generation of an immune response to the antigen or combination of the antigens in the subject.

26. The method of claim 25, wherein the target organism is Vaccinia virus.

27. A method of developing a subunit vaccine against a target organism, comprising: providing a nucleic acid sequence operably encoding a target organism antigen that has been identified as capable of eliciting an immune response through the method of claim 16; introducing the nucleic acid sequence alone or in combination with at least one other nucleic acid that is capable of expressing a target organism antigen to a subject; and monitoring the generation of an immune response to the nucleic acid or combination of nucleic acids in the subject.

28. The method of claim 27, wherein the target organism is Vaccinia virus.

29. A method of developing a subunit vaccine against a target organism, comprising: providing a nucleic acid sequence operably encoding a target organism antigen that has been identified as capable of eliciting an immune response through the method of claim 18; introducing the nucleic acid sequence alone or in combination with at least one other nucleic acid that is capable of expressing a target organism antigen to a subject; and monitoring the generation of an immune response to the nucleic acid or combination of nucleic acids in the subject.

30. The method of claim 29, wherein the target organism is Vaccinia virus.

31. An array of at least 20 target organism polypeptides prepared according to claim 1.

32. A method of screening an array of target organism polypeptides in order to identify a target organism antigen that is capable of eliciting a cell-mediated immune response, comprising: providing the array of target organism polypeptides of claim 31; delivering at least 10 of the target organism polypeptides into a plurality of antigen-presenting cells; and assaying the antigen-presenting cells with at least one T-cell from an animal that has been immunized with one or more antigens from the target organism to identify a target organism antigen capable of eliciting a cell-mediated immune response.

33. The method of claim 32, wherein the target organism is Vaccinia virus.

34. The method of claim 32, wherein the antigen-presenting cells are B cells.

35. The method of claim 32, wherein the antigen-presenting cells are macrophages.

36. The method of claim 32, wherein the antigen-presenting cells are dendritic cells.

37. A method of developing a subunit vaccine against a target organism, comprising: providing a target organism antigen that is capable of eliciting an immune response, identified according to the method of claim 32; administering the antigen alone or in combination with at least one other target organism antigen that is capable of eliciting an immune response to a subject; and monitoring the generation of an immune response to the antigen or combination of the antigens in the subject.

38. The method of claim 37, wherein the target organism is Vaccinia virus.

39. A method of developing a subunit vaccine against a target organism comprising: providing a nucleic acid sequence operably encoding a target organism antigen that has been identified as capable of eliciting an immune response through the method of claim 32; introducing the nucleic acid sequence alone or in combination with at least one other nucleic acid that is capable of expressing a target organism antigen to a subject; and monitoring the generation of an immune response to the nucleic acid or combination of nucleic acids in the subject.

40. The method of claim 39, wherein the target organism is Vaccinia virus.

41. An array of at least 20 target organism polypeptides attached to a linker molecule prepared according to claim 7.

42. A method of screening an array of target organism polypeptides in order to identify a target organism antigen that is capable of eliciting a humoral immune response, comprising: providing an array of target organism virus polypeptides attached to a linker molecule prepared according to the method of claim 41; immobilizing at least 10 of the target organism polypeptides to a solid support; and assaying the polypeptides with at least one antibody from an animal that has been immunized with one or more antigens from a target organism to identify a target organism antigen capable of eliciting a humoral immune response.

43. The method of claim 42, wherein the target organism is Vaccinia virus.

44. A method of developing a subunit vaccine against a target organism, comprising: providing a target organism antigen that is capable of eliciting an immune response, identified according to the method of claim 42; administering the antigen alone or in combination with at least one other target organism antigen that is capable of eliciting an immune response to a subject; and monitoring the generation of an immune response to the antigen or combination of the antigens in the subject.

45. The method of claim 44, wherein the target organism is Vaccinia virus.

46. A method of developing a subunit vaccine against a target organism, comprising: providing a nucleic acid sequence operably encoding a target organism antigen that has been identified as capable of eliciting an immune response through the method of claim 42; introducing the nucleic acid sequence alone or in combination with at least one other

a subject; and monitoring the generation of an immune response to the nucleic acid or combination of nucleic acids in the subject.

47. The method of claim 46, wherein the target organism is *Vaccinia* virus.

48. An automated system capable of performing the method of claim 1.

49. An array comprising a plurality of individual locations, wherein a different polypeptide from a target organism is positioned at each location, and wherein at least about 50% of all expressed polypeptides from the target organism are positioned on the array.

50. The array of claim 49, wherein at least about 75% of all expressed polypeptides from the target organism are positioned on the array.

51. The array of claim 49, wherein about 100% of all expressed polypeptides from the target organism are positioned on the array.

52. The array of claim 49, wherein the target organism is *Vaccinia* virus.

53. The array of claim 49, wherein the positioned polypeptides are attached to a linker molecule, selected from the group consisting of: a 6x, 7x, 8x, 9x, or 10x histidine tag, GST tag, fluorescent protein tag, and Flag tag.

54. The array of claim 53, wherein the positioned polypeptides are bound to the individual locations.

55. The array of claim 49, wherein the array comprises a plurality of sub-arrays.

56. The array of claim 55, wherein the sub-arrays are microtiter plates.

57. The array of claim 56, wherein the microtiter plates are 96-well plates.

58. A method of generating a library of target organism polypeptides, comprising the following steps: (a) **PCR** cloning a desired nucleic acid coding sequence from the target organism into a vector by flanking the coding sequence with first and second adapter sequences, wherein the first and second adapter sequences, wherein the first and second adapter sequences are added by **PCR**; (b) contacting the coding sequence with the vector having sequences homologous to the first and second adapter sequences within a host cell under conditions such that the coding sequence is incorporated into the vector by recombination in vivo in the host cell. (c) expressing the polypeptide encoded by the coding sequence; and (d) repeating steps (a)-(c) at least 10 times, with different coding sequences to express different polypeptides of said target organism.

59. The method of claim 58, wherein steps (a)-(d) are repeated about 266 times.

60. The method of claim 58, wherein the target organism is *Vaccinia* virus.

61. The method of claim 58, wherein the target organism is *B. anthracis*.

62. The method of claim 58, wherein the target organism is *Francisella tularensis*.

63. The method of claim 58, wherein the target organism is *P. falciparum*.

64. The method of claim 58, wherein the target organism is *Mycobacterium tuberculosis*.

65. The method of claim 58, further comprising adding at least one polynucleotide sequence operably encoding a linker molecule to the nucleic acid coding sequence from the target organism, wherein the linker molecule immobilizes the expressed polypeptide to a solid support.

66. The method of claim 65, wherein expressing the desired nucleic acid coding sequence and the polynucleotide sequence operably encoding a linker molecule produces a target organism polypeptide attached to a linker molecule.

67. A method of screening a library of target organism polypeptides in order to identify a target organism antigen that is capable of eliciting

organism polypeptides attached to a linker molecule prepared according to the method of claim 66; immobilizing at least 10 of the target organism polypeptides to a solid support; and assaying the polypeptides with at least one antibody from an animal that has been immunized with one or more antigens from the target organism to identify a target organism antigen capable of eliciting a humoral immune response.

68. The method of claim 67, wherein the target organism is *Vaccinia* virus.

69. The method of claim 67, wherein the target organism is *B. anthracis*.

70. The method of claim 67, wherein the target organism is *Francisella tularensis*.

71. The method of claim 67, wherein the target organism is *P. falciparum*.

72. The method of claim 67, wherein the target organism is *Mycobacterium tuberculosis*.

73. A method of screening a library of target organism polypeptides in order to identify a target organism antigen that is capable of eliciting a cell-mediated immune response, comprising: providing a library of target organism polypeptides according to the method of claim 58; delivering at least 10 of the target organism polypeptides into a plurality of antigen-presenting cells; and assaying the antigen-presenting cells with at least one T-cell from an animal that has been immunized with one or more antigens from the target organism to identify a target organism antigen capable of eliciting a cell-mediated immune response

74. The method of claim 73, wherein the target organism is *Vaccinia* virus.

75. The method of claim 73, wherein the target organism is *B. anthracis*.

76. The method of claim 73, wherein the target organism is *Francisella tularensis*.

77. The method of claim 73, wherein the target organism is *P. falciparum*.

78. The method of claim 73, wherein the target organism is *Mycobacterium*.

79. A method of generating a library of target organism polypeptides, comprising the following steps: (a) amplifying a desired polynucleotide coding sequence from the target organism by performing **PCR** with a first **primer** pair capable of amplifying the desired polynucleotide coding sequence; (b) expressing the amplified polynucleotide coding sequence; and (c) repeating steps (a)-(b) at least 10 times, with different first **primer** pairs to express different polypeptides of said target organism.

80. The method of claim 79, wherein the **PCR** reaction comprises: (a) performing a first **PCR** reaction using a first **primer** pair capable of amplifying a desired polynucleotide sequence from the target organism to provide an amplified coding sequence, which amplified coding sequence is not transcriptionally active; (b) providing a second **PCR** nucleotide **primer** pair capable of adding at least one nucleotide sequence that confers transcriptional activity to the amplified coding sequence; (c) performing a second **PCR** reaction with the second **primer** pair and the amplified coding sequence, thereby resulting in amplification of a transcriptionally active coding sequence.

L5 ANSWER 60 OF 112 USPATFULL on STN

2003:120056 Secondary structure defining database and methods for determining identity and geographic origin of an unknown bioagent thereby.

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US 2003082539 A1 20030501

APPLICATION: US 2001-891793 A1 20010626 (9)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates generally to the field of investigational bioinformatics and more particularly to secondary structure defining

interrogating a database as a source of molecular masses of known bioagents for comparing against the molecular mass of an unknown or selected bioagent to determine either the identity of the selected bioagent, and/or to determine the origin of the selected bioagent. The identification of the bioagent is important for determining a proper course of treatment and/or eradication of the bioagent in such cases as biological warfare. Furthermore, the determination of the geographic origin of a selected bioagent will facilitate the identification of potential criminal identity.

CLM What is claimed is:

1. A method of identifying an unknown bioagent using a database of molecular masses of known bioagents comprising: contacting nucleic acid from said bioagent with at least one pair of **oligonucleotide primers** that hybridize to sequences of said nucleic acid, wherein said sequences flank a variable nucleic acid sequence of said bioagent; producing an amplification product of said variable nucleic acid sequence; determining a first molecular mass of said amplification product; and comparing said first molecular mass to the molecular masses of known bioagents, thereby identifying the unknown bioagent.

2. The method of claim 1 wherein said sequences to which said at least one pair of **oligonucleotide primers** hybridize are highly conserved.

3. The method of claim 1 wherein said sequences to which said at least one pair of **oligonucleotide primers** hybridize are highly conserved across at least two species.

4. The method of claim 1 further comprising the step of isolating a nucleic acid from said bioagent prior to contacting said nucleic acid with said at least one pair of **oligonucleotide primers**, wherein the comparing step further comprises comparing a base-pair count resulting from a translation of the corresponding molecular mass, and wherein a master database of molecular masses of known bioagents further includes a translation of said molecular masses of known bioagents to corresponding base-pair counts of each known bioagent resulting from a specific **primer** pair set and comparing the base-pair count of said unknown bioagent against the obtained base-pair count of known bioagents for the selected **primer** pair set for determining the identity of said unknown bioagent.

5. The method of claim 4 further comprising the step of reconciling the database of molecular masses of known bioagents with the master database of molecular masses of known bioagents.

6. The method of claim 1 wherein said bioagent is a bacterium, virus, cell or spore.

7. The method of claim 1 wherein said amplification product is ionized by electrospray ionization, matrix assisted laser desorption or fast atom bombardment prior to molecular mass determination.

8. The method of claim 1 wherein said molecular mass is determined by mass spectrometry.

9. The method of claim 5 wherein said master database of molecular masses of known bioagents and the database of molecular masses of known bioagents are reconciled over a network.

10. The method of claim 4 wherein the identity is determined by statistically correlating the molecular mass of the unknown bioagent with at least one molecular mass of said master database.

11. A database having cell-data positional significance comprising at least a first table of a plurality of data-containing cells, said first table organized into at least a first row and a second row, each row having columns and data-containing cells; and wherein said data-containing cells have an alignment with at least one other row for differentiating aligned from non-aligned data-containing cells, and wherein said differentiation in alignment of said data-containing cells designates a structural feature of a polymer.

12. The database according to claim 11 wherein said alignment is a vertical alignment according to base pair homology along a linear segment within each polymer.

13. The database according to claim 11 wherein said vertical alignment further aligns cell-data according to inter-species conserved regions.

14. The database according to claim 11 wherein the structural feature is a bulge or a loop.

15. The database according to claim 11 wherein the polymer is an RNA.

16. A method for reconciling a first file and a second file, said second file corresponding at least in part to said first file, said first file and said second file each containing records, said records corresponding to rows in a table of a dimensional database having rows and columns defined by data-cells having data-cell positional significance, said method comprising: comparing said first file and said corresponding second file with a backup file containing records from a previous reconciliation of said first file and said corresponding second file to identify new, updated or deleted records; creating a reconcile file containing information pertaining to said new, updated or deleted records identified in said comparing step; and copying the contents of said reconcile file to said first file, said corresponding second file and a new backup file.

17. A service providing information related to a bioagent comprising: providing a dimensional master database for storing a molecular mass, an identity and a detail corresponding to a plurality of known bioagents and, said master database storing the molecular mass, the identity and the detail for a plurality of known bioagents; interrogating the master database with an identification request of an unknown bioagent to generate a response; and delivering said response from the master database to a requester.

18. The service according to claim 17 wherein the molecular mass is of a selected portion of the known bioagent, the identity comprises at least a geographic origin and a name for the known bioagent, and the detail comprises at least a treatment.

19. The service according to claim 17 wherein the request comprises a symptomatology and the identification comprises a recommended pair of **primers** for hybridizing to sequences of nucleic acid flanking a variable nucleic acid sequence of the unknown bioagent, and said pair of **primers** are hybridized to the sequences of nucleic acid flanking a variable nucleic acid sequence of the unknown bioagent.

20. The service according to claim 19 wherein the nucleic acid sequence of the unknown bioagent between said pair of **primers** defines the selected portion of both the known bioagents and the unknown bioagent.

21. The service according to claim 20 wherein the response is delivered through a network.

22. The service according to claim 20 wherein the request comprises a molecular mass of the unknown bioagent for the selected portion and where the response generated thereto comprises a set of molecular masses for analogous selected portions of known bioagents, and said set comprising at least one molecular mass from the master database.

23. The service according to claim 21 wherein the network is a local area network.

24. The service according to claim 21 wherein the network is a wide area network.

25. The service according to claim 22 wherein the network is the internet.

26. A method of determining a geographical origin of a selected bioagent using a database of molecular masses of known bioagents comprising: contacting nucleic acid from said selected bioagent with at least one pair of **oligonucleotide primers** which hybridize to sequences of said nucleic acid, wherein said sequences flank a variable nucleic acid sequence of the bioagent; producing an amplification product of said variable nucleic acid sequence; determining a first molecular mass of said amplification product; and comparing said first molecular mass to the molecular masses of known bioagents for determining a geographic origin of said selected bioagent, said comparison determining an identity and a geographic origin of said selected bioagent.

27. The method of claim 26 wherein said sequences to which said at least one pair of **oligonucleotide primers** hybridize are highly conserved.

28. The method of claim 26 wherein said sequences to which said at least one pair of **oligonucleotide primers** hybridize are highly conserved across species.

29. The method of claim 26 further comprising the step of isolating a nucleic acid from said selected bioagent prior to contacting said nucleic acid with said at least one pair of **oligonucleotide primers**, wherein the comparing step further comprises interrogating a master database of molecular masses of known bioagents for obtaining molecular masses of known bioagents and comparing the molecular mass of said

bioagents thereby determining an origin of said selected bioagent.

30. The method of claim 29 further comprising the step of reconciling the database of molecular masses of known bioagents with the master database of molecular masses of known bioagents.

31. The method of claim 26 wherein said bioagent is a bacterium, virus, cell or spore.

32. The method of claim 26 wherein said amplification product is ionized by electrospray ionization, matrix assisted laser desorption or fast atom bombardment prior to molecular mass determination.

33. The method of claim 26 wherein said molecular mass is determined by mass spectrometry.

34. The method of claim 29 wherein said master database of molecular masses of known bioagents and the database of molecular masses of known bioagents are reconciled over a network.

35. The method of claim 29 wherein the origin comprises a statistical group of matching molecular masses and the geographic origin corresponding thereto.

L5 ANSWER 61 OF 112 USPATFULL on STN

2003:94733 Transgenic animals and cell lines for screening drugs effective for the treatment or prevention of Alzheimer's Disease.

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US 2003066097 A1 20030403

APPLICATION: US 2001-964678 A1 20010928 (9)

PRIORITY: US 1997-38908P 19970226 (60)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Disclosed are transgenic animals and transfected cell lines expressing a protein associated with Alzheimer's Disease, neuroectodermal tumors, malignant astrocytomas, and glioblastomas. Also disclosed is the use of such transgenic animals and transfected cell lines to screen potential drug candidates for treating or preventing Alzheimer's disease, neuroectodermal tumors, malignant astrocytomas, and glioblastomas. The invention also relates to new antisense oligonucleotides, ribozymes, triplex forming DNA and external guide sequences that can be used to treat or prevent Alzheimer's disease, neuroectodermal tumors, malignant astrocytomas, and glioblastomas.

CLM What is claimed is:

1. A DNA construct, which comprises a DNA molecule of Seq. ID No. 1 or a DNA molecule which is at least 40% homologous thereto, or a fragment thereof, wherein said DNA molecule is under control of a heterologous neuro-specific promoter.

2. The DNA construct of claim 1, which is contained within a vector.

3. The DNA construct of claim 1, which is contained by a viron.

4. The DNA construct of claim 1, wherein said DNA molecule has Seq. ID No. 1.

5. A host cell transformed with the DNA construct of claim 1.

6. The host cell line of claim 5, which is a neuronal cell.

7. A transgenic non-human animal, all of whose germ and somatic cells comprises the DNA molecule of Seq. ID No. 1 or a DNA molecule which is at least 40% homologous thereto.

8. The transgenic non-human animal of claim 7, wherein the DNA molecule contained in each germ and somatic cell has Seq. ID No. 1.

9. The transgenic non-human animal of claim 7, wherein the protein coded for by said DNA molecule is overexpressed in the brain of the animal.

10. An in vitro method for screening a candidate drug that is potentially useful for the treatment or prevention of Alzheimer's disease, neuroectodermal tumors, malignant astrocytomas, and glioblastomas, which comprises (a) contacting a candidate drug with the host cell line of claim 5, and (b) detecting at least one of the following: (i) the suppression or prevention of expression of the protein coded for by the DNA construct; (ii) the increased degradation of the protein coded for by the DNA construct; or (iii) the reduction of frequency of at least one of neuritic sprouting, nerve cell death, degenerating neurons, neurofibrillary tangles, or irregular swollen

control cell line which has not contacted the candidate drug.

11. The method of claim 10, wherein said protein has Seq. ID No. 2.

12. The method of claim 10, wherein said protein is over-expressed by said host cell.

13. The method of claim 10, wherein said cell is a neuronal cell.

14. An in vivo method for screening a candidate drug that is potentially useful for the treatment or prevention of Alzheimer's disease, neuroectodermal tumors, malignant astrocytomas, and glioblastomas, which comprises (a) administering a candidate drug to the transgenic animal of claim 7, and (b) detecting at least one of the following: (i) the suppression or prevention of expression of the protein coded for by the DNA construct contained by said animal; (ii) the increased degradation of the protein coded for by the DNA construct contained by said animal; or (iii) the reduction of frequency of at least one of neuritic sprouting, nerve cell death, degenerating neurons, neurofibrillary tangles, or irregular swollen neurites and axons in the host; due to the drug candidate compared to a control animal which has not received the candidate drug.

15. The method of claim 14, wherein the DNA construct contained by said animal has Seq. ID No. 1.

16. The method of claim 14, wherein the protein coded for by the DNA construct contained by said animal is over-expressed in the brain of said animal.

17. An antisense **oligonucleotide** which is complementary to an NTP mRNA sequence corresponding to nucleotides 150-1139 of Seq. ID No. 1.

18. The antisense **oligonucleotide** of claim 17, which is a 15 to 40-mer.

19. The antisense **oligonucleotide** of claim 17, wherein said antisense **oligonucleotide** is selected from the group consisting of Seq ID Nos. 9 to 11.

20. The antisense **oligonucleotide** of claim 17, which is deoxyribonucleic acid.

21. The antisense **oligonucleotide** of claim 17, which is a deoxyribonucleic acid phosphorothioate.

22. The antisense oligonucleotide of claim 17, which is a derivative of a deoxyribonucleic acid or a deoxyribonucleic acid phosphorothioate.

23. A pharmaceutical composition comprising the antisense **oligonucleotide** of claim 17 and a pharmaceutically acceptable carrier.

24. A ribozyme comprising a target sequence which is complementary to an NTP mRNA sequence corresponding to nucleotides 150-1139 of Seq. ID No. 1.

25. A pharmaceutical composition comprising the ribozyme of claim 24 and a pharmaceutically acceptable carrier.

26. An oligodeoxynucleotide that forms triple stranded regions with the a region of AD7c-NTP coding nucleic acid and having the sequence 3'X5'-L-5'X3', wherein X comprises an AD7c-NTP nucleic acid sequence corresponding to nucleotides 150-1139 of Seq. ID No. 1, and wherein L represents an **oligonucleotide** linker or a bond.

27. A pharmaceutical composition comprising the oligodeoxynucleotide of claim 26 and a pharmaceutically acceptable carrier.

28. An oligodeoxynucleotide that forms triple stranded regions with the a region of AD7c-NTP coding nucleic acid and having the sequence 5'X3'-L-3'X5', wherein X comprises an AD7c-NTP nucleic acid sequence corresponding to nucleotides 150-1139 of Seq. ID No. 1, and wherein L represents an **oligonucleotide** linker or a bond.

29. A pharmaceutical composition comprising the oligodeoxynucleotide of claim 28 and a pharmaceutically acceptable carrier.

30. A ribonucleotide external guide nucleic acid molecule, comprising, a 10-mer nucleotide sequence corresponding to nucleotides 150-1139 of Seq. ID No. 1 fused to a 3'NCCA nucleotide sequence, wherein N is a purine.

31. The ribonucleotide external guide nucleic acid molecule of claim 30 which is selected from the group consisting of any one of Seq. ID Nos.

32. A pharmaceutical composition comprising the ribonucleotide of claim 30 and a pharmaceutically acceptable carrier.

33. A method for to treat or prevent dementias of the Alzheimer's type of neuronal degeneration; or to treat or prevent neuroectodermal tumors, malignant astrocytomas, or glioblastomas, comprising administering to an animal in need thereof an antisense **oligonucleotide**, a ribozyme, a triple helix-forming **oligonucleotide** or an ribonucleotide external guide sequence of any one of claims 17, 24, 26, 28, or 30.

34. The method of claim 32, wherein said antisense **oligonucleotide**, ribozyme, triple helix-forming **oligonucleotide** or ribonucleotide external guide sequence is administered to said animal as part of a pharmaceutically acceptable carrier.

L5 ANSWER 62 OF 112 USPATFULL on STN

2003:86817 Immune modulation method using steroid compounds.

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US 2003060425 A1 20030327

APPLICATION: US 2001-820483 A1 20010329 (9)

PRIORITY: US 1998-109924P 19981124 (60)

US 1999-140028P 19990616 (60)

US 1998-109923P 19981124 (60)

US 1999-126056P 19991019 (60)

US 1999-124087P 19990311 (60)

US 1998-110127P 19981127 (60)

US 1999-161453P 19991025 (60)

US 1999-145823P 19990727 (60)

US 1999-137745P 19990603 (60)

US 1998-112206P 19981215 (60)

US 2000-257071P 20001220 (60)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention provides compositions comprising formula 1 steroids, e.g., 16 α -bromo-3 β -hydroxy-5 α -androstan-17-one hemihydrate and one or more excipients, including compositions that comprise a liquid formulation comprising less than about 3% v/v water. The compositions are useful to make improved pharmaceutical formulations. The invention also provides methods of intermittent dosing of steroid compounds such as analogs of 16 α -bromo-3 β -hydroxy-5 α -androstan-17-one and compositions useful in such dosing regimens. The invention further provides compositions and methods to inhibit pathogen replication, ameliorate symptoms associated with immune dysregulation and to modulate immune responses in a subject using the compounds. The invention also provides methods to make and use these immunomodulatory compositions and formulations.

CLM What is claimed is:

1. A method to modulate an immune response or cellular response in a subject in need thereof comprising administering to the subject, or delivering to the subject's tissues, an effective amount of a compound of formula 1 ##STR90## wherein, each R¹, R², R³, R⁴, R⁵, R⁶ and R¹⁰ independently are --H, --OR^{PR}, --SR^{PR}, --N(R^{PR})₂, --O--Si--(R¹³)₃, --CHO, --CHS, --CH.dbd.NH, --CN, --SCN, --NO₂, --OSO₃H, --OPO₃H, an ester, a thioester, a phosphoester, a phosphothioester, a phosphonoester, a phosphiniester, a sulfite ester, a sulfate ester, an amide, an amino acid, a peptide, an ether, a thioether, an acyl group, a thioacyl group, a carbonate, a carbamate, a thioacetal, a halogen, an optionally substituted alkyl group, an optionally substituted alkenyl group, an optionally substituted alkynyl group, an optionally substituted aryl moiety, an optionally substituted heteroaryl moiety, an optionally substituted heterocycle, an optionally substituted monosaccharide, an optionally substituted oligosaccharide, a nucleoside, a nucleotide, an **oligonucleotide**, a polymer, or, one or more of both R¹, R², R³ or R⁴ together comprise an independently selected spiro ring, or one more of R¹, R², R³, R⁴, R⁵, R⁶ and R¹⁰ are .dbd.O, .dbd.S, .dbd.N--OH, .dbd.CH₂ or a spiro ring and the hydrogen atom or the second variable group that is bonded to the same carbon atom is absent, or, one or more of two adjacent R¹-R⁶ and R¹⁰ comprise an independently selected ketal or thioketal, or all R³ and R⁴ together comprise a structure of formula 2 ##STR91## R⁷ is

--C(R¹⁰)₂--C(R¹⁰)₂--C(R¹⁰)₂--,
 --C(R¹⁰)₂--O--C(R¹⁰)₂--, --C(R¹⁰)₂--S--
 C(R¹⁰)₂--, --C(R¹⁰)₂--NR^{PR}--C(R¹⁰)₂--
 , --O-- , --O--C(R¹⁰)₂--, --S-- , --S--C(R¹⁰)₂--,
 --NR^{PR}-- or --NR^{PR}--C(R¹⁰)₂--; R⁸ and R⁹
 independently are --C(R¹⁰)₂--, --C(R¹⁰)₂--
 C(R¹⁰)₂--, --O-- , --O--C(R¹⁰)₂--, --S-- ,
 --S--C(R¹⁰)₂--, --NR^{PR}-- or --NR^{PR}--
 C(R¹⁰)₂--, or one or both of R⁸ or R⁹ independently
 are absent, leaving a 5-membered ring; R¹³ independently is
 C₁₋₆ alkyl; R^{PR} independently is --H or a protecting group;
 D is a heterocycle or a 4-, 5-, 6- or 7-membered ring that comprises
 saturated carbon atoms, wherein 1, 2 or 3 ring carbon atoms of the 4-,
 5-, 6- or 7-membered ring are optionally independently substituted with
 --O-- , --S-- or --NR^{PR}-- or where 1, 2 or 3 hydrogen atoms of the
 heterocycle or where 1, 2 or 3 hydrogen atoms of the 4-, 5-, 6- or
 7-membered ring are substituted with --OR^{PR}, --SR^{PR},
 --N(R^{PR})₂, --O--Si--(R¹³)₃, --CHO, --CHS,
 --CH.dbd.NH, --CN, --SCN, --NO₂, --OSO₃H, --OPO₃H, an
 ester, a thioester, a phosphoester, a phosphothioester, a
 phosphiniester, a sulfite ester, a sulfate ester, an amide, an amino
 acid, a peptide, an ether, a thioether, an acyl group, a thioacyl group,
 a carbonate, a carbamate, a thioacetal, a halogen, an optionally
 substituted alkyl group, an optionally substituted alkenyl group, an
 optionally substituted alkynyl group, an optionally substituted aryl
 moiety, an optionally substituted heteroaryl moiety, an optionally
 substituted heterocycle, an optionally substituted monosaccharide, an
 optionally substituted oligosaccharide, a nucleoside, a nucleotide, an
oligonucleotide or a polymer, or, one more of the ring carbons are
 substituted with .dbd.O, .dbd.S, .dbd.N--OH, .dbd.CH₂, or a spiro
 ring, or D comprises two 5- or 6-membered rings, wherein the rings are
 fused or are linked by 1 or 2 bonds, but provided the formula 1 compound
 is not 4-pregnene-11β, 17α, 21-triol-3,20-dione, 17α,
 21-dihydroxypregn-4-ene-3,11,20-trione, 11β, 21-dihydroxy-3,20-
 dioxopregn-4-en-18-al, 11 β, 17α, 21-trihydroxypregna-1,4-
 diene-3,20-dione, 17α, 21-dihydroxypregna-1,4-diene-3,11,
 20-trione, 3β-hydroxypregn-5-ene-20-one, 3β-hydroxyandrost-5-
 ene-17-one, pregn-4-ene-3,20-dione, 21-hydroxypregn-4-ene-3, 20-dione,
 9-fluoro-11 β, 16α, 21-trihydroxy-16-methylpregna-1,4-diene-
 3,20-dione, 9-fluoro-11β, 16α, 17,21-tetrahydroxypregna-1,4-
 diene-3,20-dione, 9-fluoro-11β, 17α, 21-trihydroxy-16-
 methylpregna-1,4-diene-3,20-dione or an ester or ether of any of these,
 and provided that one or more of the following apply (1) the formula 1
 compound is administered to the subject, or delivered to the subject's
 tissues using an intermittent dosing protocol, or (2) the formula 1
 compound is a component in a liquid formulation that comprises less than
 about 3% v/v of water and one or two of R⁷, R⁸ or R⁹
 independently are --O-- , --S-- or --NH-- , or at least one of
 R^{1-R6} and R¹⁰ is an amino acid, or a double bond is
 present at the 1-2 position, 4-5 position or at the 16-17 position, or
 at least one of R^{1-R4} independently is a --SH, a halogen, or a
 carbamate, or (3) the formula 1 compound is a component in a buccal or
 sublingual formulation that optionally comprises one or more of sucrose,
 mannitol, povidone and magnesium stearate; or (4) one or two of
 R⁷, R⁸ or R⁹ independently are --O-- , --S-- or --NH-- ,
 or (5) at least one of R^{1-R6} and R¹⁰ is an amino acid,
 or (6) a double bond is present at the 1-2 position, 4-5 position or at
 the 16-17 position.

2. The method of claim 1 wherein the immune response or the cellular
 response is an enhanced Th1 immune response, a reduced Th2 immune
 response, reduced inflammation, enhanced hemopoiesis, inhibited tumor
 cell proliferation, inhibited replication of a pathogen or amelioration
 of a symptom of any of these conditions.

3. The method of claim 2 wherein the reduced inflammation is in a
 subject suffering from an autoimmune condition or an inflammation
 condition.

4. The method of claim 3 wherein the autoimmune condition or the
 inflammation condition is rheumatoid arthritis, osteoarthritis, systemic
 lupus erythematosus, asthma, multiple sclerosis, an infection or
 vascular inflammation.

5. A composition comprising a compound of formula 1 ##STR92## and one
 or more nonaqueous liquid excipients, wherein the composition comprises
 less than about 3% v/v water and wherein, R¹, R², R³,
 R⁴, R⁵, R⁶ and R¹⁰ independently are --H,
 --OR^{PR}, --SR^{PR}, --N(R^{PR})₂, --O--Si--
 (R¹³)₃, --CHO, --CHS, --CH.dbd.NH, --CN, --SCN, --NO₂,

phosphothioester, a phosphonoester, a phosphiniester, a sulfite ester, a sulfate ester, an amide, an amino acid, a peptide, an ether, a thioether, an acyl group, a thioacyl group, a carbonate, a carbamate, a thioacetal, a halogen, an optionally substituted alkyl group, an optionally substituted alkenyl group, an optionally substituted alkynyl group, an optionally substituted aryl moiety, an optionally substituted heteroaryl moiety, an optionally substituted monosaccharide, an optionally substituted oligosaccharide, a nucleoside, a nucleotide, an **oligonucleotide**, a polymer, or, one, two or more of R¹, R², R³, R⁴, R⁵, R⁶ and R¹⁰ independently are

.dbd.O, .dbd.S, .dbd.N--OH, .dbd.CH₂ or a spiro ring and the hydrogen atom or second variable group that is bonded to the same carbon atom is absent, or, one or more of two adjacent R¹-R⁶ and R¹⁰ comprise an independently selected ketal orthioketal, or R³ and both R⁴ together comprise a structure of formula 2

##STR93## R⁷ is --C(R¹⁰)₂--, --C(R¹⁰)₂--

C(R¹⁰)₂--, --C(R¹⁰)₂--C(R¹⁰)₂--

C(R¹⁰)₂--, --C(R¹⁰)₂--O--C(R¹⁰)₂--,

--C(R¹⁰)₂--S--C(R¹⁰)₂--, --C(R¹⁰)₂--

NR^{PR}--C(R¹⁰)₂--, --O--C(R¹⁰)₂--, --S--C(R¹⁰)₂--,

--S--C(R¹⁰)₂--, --NR^{PR}-- or --NR^{PR}--

C(R¹⁰)₂--; R⁸ and R⁹ independently are

--C(R¹⁰)₂--, --C(R¹⁰)₂--C(R¹⁰)₂--, --O--C(R¹⁰)₂--,

--O--C(R¹⁰)₂--, --S--C(R¹⁰)₂--, --S--C(R¹⁰)₂--, --NR^{PR}--

or --NR^{PR}--C(R¹⁰)₂--, or one or both of R⁸ or

R⁹ independently are absent, leaving a 5-membered ring; R¹³

independently is C₁₋₆ alkyl; D is a heterocycle or a 4-, 5-, 6- or 7-membered ring that comprises saturated carbon atoms, wherein 1, 2 or 3 ring carbon atoms of the 4-, 5-, 6- or 7-membered ring are optionally independently substituted with --O--C(R¹⁰)₂--, --S--C(R¹⁰)₂-- or --NR^{PR}-- or where 1, 2 or 3 hydrogen atoms of the heterocycle or where 1, 2 or 3 hydrogen atoms of the 4-, 5-, 6- or 7-membered ring are substituted with

--OR^{PR}, --SR^{PR}, --N(R^{PR})₂, --O--Si--

(R¹³)₃, --CHO, --CHS, --CH.dbd.NH, --CN, --SCN, --NO₂,

--OSO₃H, --OPO₃H, an ester, a thioester, a phosphoester, a

phosphothioester, a phosphonoester, a phosphiniester, a sulfite ester, a sulfate ester, an amide, an amino acid, a peptide, an ether, a thioether, an acyl group, a thioacyl group, a carbonate, a carbamate, a thioacetal, a halogen, an optionally substituted alkyl group, an optionally substituted alkenyl group, an optionally substituted alkynyl group, an optionally substituted aryl moiety, an optionally substituted heteroaryl moiety, an optionally substituted monosaccharide, an optionally substituted oligosaccharide, a nucleoside, a nucleotide, an **oligonucleotide** or a polymer, or, one or more of the ring carbons are substituted with .dbd.O, .dbd.S, .dbd.N--OH, .dbd.CH₂ or a spiro ring and the hydrogen atom that is bonded to the same carbon atom is absent, or D comprises two 5- or 6-membered rings, wherein the rings are fused or are linked by 1 or 2 bonds, but provided the formula 1

compound is not 4-pregnene-11 β , 17 α , 21-triol-3, 20-dione,

17 α , 21-dihydroxypregn-4-ene-3, 11, 20-trione, 11 β ,

21-dihydroxy-3, 20-dioxopregn-4-en-18-al, 11 β , 17 α , 21-

trihydroxypregna-1, 4-diene-3, 20-dione, 17 α , 21-dihydroxypregna-1, 4-

diene-3, 11, 20-trione, 3 β -hydroxypregn-5-ene-20-one,

3 β -hydroxyandrost-5-ene-17-one, pregn-4-ene-3, 20-dione,

21-hydroxypregn-4-ene-3, 20-dione, 9-fluoro-11 β , 16 α , 21-

trihydroxy-16-methylpregna-1, 4-diene-3, 20-dione, 9-fluoro-

11 β , 16 α , 17, 21-tetrahydroxypregna-1, 4-diene-3, 20-dione,

9-fluoro-11 β , 17 α , 21-trihydroxy-16-methylpregna-1, 4-diene-3, 20-

dione or an ester or ether of any of these.

6. The composition of claim 5 wherein formula 2 has the structure

##STR94## wherein R¹⁵, R¹⁷ and R¹⁸ independently are

--H, --OR^{PR}, --SR^{PR}, --N(R^{PR})₂, --O--Si--(R

13)₃, --CHO, --CHS, --CH.dbd.NH, --CN, --SCN, --NO₂,

--OSO₃H, --OPO₃H, an ester, a thioester, a phosphoester, a

phosphothioester, a phosphonoester, a phosphiniester, a sulfite ester, a sulfate ester, an amide, an amino acid, a peptide, an ether, a thioether, an acyl group, a thioacyl group, a carbonate, a carbamate, a thioacetal, a halogen, an optionally substituted alkyl group, an optionally substituted alkenyl group, an optionally substituted alkynyl group, an optionally substituted aryl moiety, an optionally substituted heteroaryl moiety, an optionally substituted heterocycle, an optionally substituted monosaccharide, an optionally substituted oligosaccharide, a nucleoside, a nucleotide, an **oligonucleotide**, a polymer, or, one or more of R¹⁵, R¹⁷ and R¹⁸ independently are .dbd.O,

.dbd.S, .dbd.N--OH, .dbd.CH₂ or a spiro ring and the hydrogen atom

that is bonded to the same carbon atom is absent; R¹⁶

independently are --CH₂--, --O--C(R¹⁰)₂--, --S--C(R¹⁰)₂-- or --NH--; and R¹⁹ is

nitrogen or CH.

7. The composition of claim 6 wherein one, two or three of R⁷, R⁸ and R⁹ are independently --O--, --S--, or --NH-- or wherein one or both of R⁵ and R⁶ independently are --H, --CH₃, --CH₂OR^{PR}, 'CH₂SR^{PR}, --CH₂--c(O)--C₁₋₁₀ alkyl, --CH₂--c(O)--C₁₋₁₀ alkenyl, --CH₂--c(O)--C₁₋₁₀ alkenyl, --CH₂--c(O)--C₀₋₄ alkyl-heterocycle, --CH₂--c(O)--C₀₋₄ alkyl-heterocycle, --CH₂--c(O)--C₀₋₄ alkyl-phenyl, --CH₂--c(O)--C₀₋₄ alkyl-phenyl, wherein any C₁₋₁₀ alkyl, heterocycle or phenyl moiety is optionally substituted with one or more substituents.

8. The composition of claim 7 wherein the one or more substituents are one, two, three or more independently selected --O--, .dbd.O, --OR^{PR}, --S--, .dbd.S, --SR^{PR}, --NH--, --N(R^{PR})₂ or --C(O)--NH--, wherein each R^{PR} independently is --H or a protecting group.

9. The composition of claim 5 wherein R¹ and R⁴ independently are --OH, --O-alkyl, --O--C(O)-alkyl, .dbd.O, --SH, --S-alkyl, --S--C(O)-alkyl or .dbd.S, and R² and R³ is --H, --OH, --O-alkyl, --O--C(O)-alkyl, .dbd.O, --SH, --S-alkyl, --S--C(O)-alkyl or .dbd.S.

10. The composition of claim 5 wherein the formula 1 compound is a compound named in compound groups 1 through 54-53-52-51a6-50c27-49c27-48-47-46-45-44-43-42-41-40-39-38-37-36-35-34-33-32-31-30-29-28-27-39-38-37-36-35 -34-33-32-31-30-29-28-27-26-25-23-21-17-10-8-6.

11. A compound of formula 1 ##STR95## wherein, R¹, R², R³, R⁴, R⁵, R⁶ and R¹⁰ independently are --H, --OR^{PR}, --SR^{PR}, --N(R^{PR})₂, --O--Si--(R¹³)₃, --CHO, --CHS, --CH.dbd.NH, --CN, --SCN, --NO₂, --OSO₃H, --OPO₃H, an ester, a thioester, a phosphoester, a phosphothioester, a phosphonoester, a phosphiniester, a sulfite ester, a sulfate ester, an amide, an amino acid, a peptide, an ether, a thioether, an acyl group, a thioacyl group, a carbonate, a carbamate, a thioacetal, a halogen, an optionally substituted alkyl group, an optionally substituted alkenyl group, an optionally substituted alkynyl group, an optionally substituted aryl moiety, an optionally substituted heteroaryl moiety, an optionally substituted heterocycle, an optionally substituted monosaccharide, an optionally substituted oligosaccharide, a nucleoside, a nucleotide, an **oligonucleotide**, a polymer, or, one, two or more of R¹, R², R³, R⁴, R⁵, R⁶ and R¹⁰ independently are .dbd.O, .dbd.S, .dbd.N--OH, .dbd.CH₂ or a spiro ring and the hydrogen atom that is bonded to the same carbon atom is absent, or, one or more of two adjacent R¹-R⁶ and R¹⁰ comprise an independently selected ketal or thioketal; or R³ and R⁴ together comprise a structure of formula 2 ##STR96## R⁷ is --C(R¹⁰)₂--, --C(R¹⁰)₂--c(R¹⁰)₂--, --C(R¹⁰)₂--c(R¹⁰)₂--c(R¹⁰)₂--, --C(R¹⁰)₂--o--c(R¹⁰)₂--, --C(R¹⁰)₂--s--C(R¹⁰)₂--, --C(R¹⁰)₂--NR^{PR}--C(R¹⁰)₂--, --O--, --O--C(R¹⁰)₂--, --S--, --S--C(R¹⁰)₂--, --NR^{PR}-- or --NR^{PR}--C(R¹⁰)₂--; R⁸ and R⁹ independently are --C(R¹⁰)₂--, --C(R¹⁰)₂--C(R¹⁰)₂--, --O--, --O--C(R¹⁰)₂--, --S--, --S--C(R¹⁰)₂--, --NR^{PR}-- or --NR^{PR}--C(R¹⁰)₂--, or one or both of R⁸ or R⁹ independently are absent, leaving a 5-membered ring; R¹³ independently is C₁₋₆ alkyl; D is a heterocycle or a 4-, 5-, 6- or 7-membered ring that comprises saturated carbon atoms, wherein 1, 2 or 3 ring carbon atoms of the 4-, 5-, 6- or 7-membered ring are optionally independently substituted with --O--, --S-- or --NR^{PR}-- or where 1, 2 or 3 hydrogen atoms of the heterocycle or where 1 or 2 hydrogen atoms of the 4-, 5-, 6- or 7-membered ring are substituted with --OR^{PR}, --SR^{PR}, --N(R^{PR})₂, --O--Si--(R¹³)₃, --CHO, --CHS, --CH.dbd.NH, --CN, --NO₂, an ester, a thioester, a phosphoester, a phosphothioester, a phosphonoester, a phosphiniester, a sulfite ester, a sulfate ester, an amide, an amino acid, a peptide, an ether, a thioether, an acyl group, a thioacyl group, a carbonate, a carbamate, a thioacetal, a halogen, an optionally substituted alkyl group, an optionally substituted alkenyl group, an optionally substituted alkynyl group, an optionally substituted aryl moiety, an optionally substituted heteroaryl moiety, an optionally substituted monosaccharide, an optionally substituted oligosaccharide, a nucleoside, a nucleotide, an **oligonucleotide** or a polymer, or, one or more of the ring carbons are substituted with .dbd.O, .dbd.S, .dbd.N--OH, .dbd.CH₂ or a spiro ring, or D comprises two 5- or 6-membered rings, wherein the rings are fused or are linked by 1 or 2 bonds, wherein two or three of R⁷,

12. The compound of claim 11 wherein formula 2 has the structure
##STR97## wherein R¹⁵, R¹⁷ and R¹⁸ independently are
--H, --OR^{PR}, --SR^{PR}, --N(R^{PR})₂, --O--Si--
(R¹³)₃, --CHO, --CHS, --CH.dbd.NH, --CN, --SCN, --NO₂,
--OSO_{3H}, --OPO_{3H}, an ester, a thioester, a phosphoester, a
phosphothioester, a phosphonoester, a phosphiniester, a sulfite ester, a
sulfate ester, an amide, an amino acid, a peptide, an ether, a
thioether, an acyl group, a thioacyl group, a carbonate, a carbamate, a
thioacetal, a halogen, an optionally substituted alkyl group, an
optionally substituted alkenyl group, an optionally substituted alkynyl
group, an optionally substituted aryl moiety, an optionally substituted
heteroaryl moiety, an optionally substituted monosaccharide, an
optionally substituted oligosaccharide, a nucleoside, a nucleotide, an
oligonucleotide, a polymer, or, one or more of R¹⁵, R¹⁷ and
R¹⁸ independently are .dbd.O, .dbd.S, .dbd.N--OH, .dbd.CH₂ or
a spiro ring and the hydrogen atom that is bonded to the same carbon
atom is absent; R¹⁶ independently are --CH₂--, --O--, --S--
or --NH--; and R¹⁹ is nitrogen or CH.

13. The compound of claim 12 wherein no double bonds are present, or
wherein one double bond is present.

14. The compound of claim 12 wherein one, two or three of R⁷,
R⁸ and R⁹ are independently --O--, --S--, or --NH-- or wherein
one or both of R⁵ and R⁶ independently are --H, --CH₃,
--CH_{2OR}^{PR}, --CH_{2SR}^{PR}, --CHO, --CHS, --CH.dbd.NH,
--CH_{2O}--c(O)--C₁₋₁₀ alkyl, --CH_{2S}--c(O)--C₁₋₁₀
alkyl, --CH_{2O}--c(O)--C₁₋₁₀ alkenyl, --alkenyl,
--CH_{2S}--c(O)--C₁₋₁₀ alkenyl, --CH₂--c(O)--CO--₀₋₄
alkyl-heterocycle, --CH_{2S}--c(O)--CO--₀₋₄ alkyl-heterocycle,
--CH_{2O}--c(O)--CO--₀₋₄ alkyl-phenyl, --CH_{2S}--c(O)--CO--
4 alkyl-phenyl, wherein any C₁₋₁₀ alkyl, C₁₋₁₀ alkenyl,
heterocycle or phenyl moiety is optionally substituted with one or more
independently selected substituents.

15. The compound of claim 14 wherein the one or more independently
selected substituents are one, two, three or more independently selected
--O--, .dbd.O, --OR^{PR}, --S--, .dbd.S, --SR^{PR}, --NH--,
--N(R^{PR})₂ or --C(O)--NH--, wherein each R^{PR} independently
is --H or a protecting group.

16. The compound of claim 15 wherein R¹ and R⁴ independently
are --OH, --O-alkyl, --O--C(O)-alkyl, .dbd.O, --SH, --S-alkyl,
--S--C(O)-alkyl or .dbd.S, and R² and R³ independently are
--H, --OH, --O-alkyl, --O--C(O)-alkyl, .dbd.O, --SH, --S-alkyl, --S--,
--C(O)-alkyl or .dbd.S.

17. The compound of claim 11 wherein the formula 1 compound is a
compound named in compound groups 1 through 54-53-52-51a6-50c27-49c27-48-
47-46-45-44-43-42-41-40-39-38-37-36-35-34-33-32-31-30-29-28-27-39-38-37-
36-35-34-33-32-31-30-29-28-27-26-25-23-21-17-10-8-6.

18. The compound of claim 17 wherein one or two of R⁷, R⁸ and
R⁹ independently is --O--, --S-- or --NH--.

19. The compound of claim 17 wherein one or two of R⁷, R⁸ and
R⁹ independently is --O--, --S-- or --NH-- and R¹, R² and
R⁴ independently are --OH, --SH or group that can hydrolyze to --OH
or --SH.

20. The compound of claim 19 wherein R¹ is in the
α-configuration and R⁵ and R⁶ are both in the
α-configuration and R⁵ and R⁶ independently are
--CH₃ or --CH_{2OH} or a group that can hydrolyze to
--CH_{2OH}.

21. The compound of claim 20 wherein R² is in the
α-configuration and any hydrogen at the 5-position is in the
α-configuration.

22. The compound of claim 21 wherein the formula 1 compound is an analog
of 16α-bromo-3β-hydroxy-5α-androstan-17-one,
16α-bromo-3β,7β-dihydroxy-5α-androstan-17-one,
16α-bromo-3β,7β,17β-trihydroxy-5α-androstane,
16α-bromo-3β,7α-dihydroxy-5α-androstan-17-one,
16α-bromo-3β,7α,17β-trihydroxy-5α-
androstan-17-one, 16α-bromo-3α,7β-dihydroxy-5α-
androstan-17-one, 16α-bromo-3β,7β,17β-trihydroxy-

[illegible]

16 α -bromo-7 α -hydroxy-1-androstene-3,17-dione,
3 β -hydroxy-1-androstene-17-one, 3 β ,7 β -dihydroxy-1-
androstene-17-one, 3 β ,7 β ,17 β -trihydroxy-1-androstene,
3 β ,7 α -dihydroxy-1-androstene-17-one, 3 β ,7 β ,17 β -
trihydroxy-1-androstene, 3 α ,7 β -dihydroxy-1-androstene-17-one,
3 β ,7 β ,17 α -trihydroxy-1-androstene, 3 α ,7 α -
dihydroxy-1-androstene-17-one, 3 β ,7 α ,17 α -trihydroxy-1-
androstene, 3 β -hydroxy-1-androstene-7,17-dione,
3 α -hydroxy-1-androstene-7,17-dione, 7 β -hydroxy-1-androstene-
3,17-dione or 7 α -hydroxy-1-androstene-3,17-dione, wherein one, two
or three independently selected moieties hydrolyzable to a hydroxyl
group are optionally bonded to one, two or three hydroxyl or thiol
groups, and wherein one or two of R⁷, R⁸ and R⁹ are not
--C(R¹⁰)₂--.

23. A composition comprising a compound of claim 22, and one or more excipients.

24. The composition of claim 23 wherein the one or excipients is a solid excipient, a nonaqueous liquid excipient or water.

25. The composition of claim 24, wherein the composition is a unit dosage formulation that comprises about 5 to about 1000 mg of one formula 1 compound.

26. A product produced by the process of contacting the compound of claim 11, and one or more excipients suitable for human pharmaceutical use or for veterinary use.

27. The product of claim 26 wherein the excipient is a solid excipient, a nonaqueous liquid excipient or water.

28. The product of claim 27, wherein the product is a unit dosage formulation that comprises about 3.5 mg to about 1000 mg of one formula 1 compound.

29. A method comprising administering to a subject or delivering to the subject's tissues an effective amount of a composition of claim 11 wherein the subject has or is susceptible to a pathogen infection or a malignancy, whereby the pathogen infection or the malignancy is prevented or delayed or whereby one or more symptoms of the pathogen infection or the malignancy is ameliorated or whereby the replication of a pathogen primarily responsible for the pathogen infection is inhibited or whereby replication of the malignant cells is inhibited, or whereby at least a portion of an extracellular pathogen primarily responsible for the pathogen infection, or cells infected by the pathogen or the malignant cells are killed, or whereby the malignant cell population is reduced, or whereby the degree of pathogen replication is reduced.

30. The method of claim 29 further comprising intermittently administering to the subject or delivering to the subject's tissues an effective amount of the composition of claim 11.

31. The method of claim 30 wherein (a) the composition of claim 11 is administered to the subject or delivered to the subject's tissues (i) one, two or three times per day for 1, 2, 3, 4 or 5 consecutive days or (ii) one, two or three times per day every other day over a period of 3, 5, 7 or 9 days; (b) not administering the composition of claim 15 for at least about 28 days to at least about 8 months from the last day of dosing in step (a); (c) administering to the subject or delivering to the subject's tissues the composition of claim 15 (i) one, two or three times per day for 1, 2, 3, 4 or 5 consecutive days or (ii) one, two or three times per day every other day over a period of 3, 5, 7 or 9 days; and (d) optionally repeating steps (a), (b) and (c) 1, 2, 3, 4, 5 or more times.

32. The method of claim 30 wherein (a) the composition of claim 15 is administered to the subject or delivered to the subject's tissues once per day every other day over a period of 5 days; (b) not administering the composition of claim 15 for 2 days; (c) and then repeating step (a); and (d) optionally repeating steps (a), (b) and (c) 1, 2, 3 or more times.

33. The method of claim 32 wherein about 0.05 mg/kg to about 25 mg/kg of the formula 1 compound that is present in the composition of claim 15 is administered to the subject or delivered to the subject's tissues.

34. The method of claim 1 wherein the modulation is an enhanced Th1 immune response or a reduced Th2 immune response.

immunosuppression condition (a suboptimal Th1 immune response) or an unwanted immune response (excess Th2 immune response), either or both of which are associated with (1) a pathogen infection selected from a viral infection, an intracellular bacterial infection, an extracellular bacterial infection, a fungal infection, a yeast infection, an extracellular parasite infection, an intracellular parasite infection, a protozoan parasite infection and a multicellular parasite infection, (2) an autoimmune disease, (3) a malignancy or a precancer, (4) a chemotherapy, a radiation therapy, an immunosuppressive therapy, an anti-infective agent therapy, a wound, a burn, the presence of an immunosuppressive molecule, or gastrointestinal irritation or an inflammation condition optionally selected from or associated with irritable bowel disease, Crohn's disease or chronic diarrhea or (5) any combination of the foregoing.

36. The method of claim 35 wherein the subject's immunosuppression condition is ameliorated or the unwanted immune response is reduced.

37. The method of claim 36 wherein the subject's innate immunity, specific immunity or both is enhanced.

38. The method of claim 35 wherein the subject's viral infection, intracellular bacterial infection, extracellular bacterial infection, fungal infection, yeast infection, extracellular parasite infection, intracellular parasite infection, protozoan parasite, multicellular parasite, autoimmune disease, cancer, precancer, chemotherapy, radiation therapy, immunosuppressive therapy, anti-infective agent therapy, a wound, a burn, or the presence of an immunosuppressive molecule, gastrointestinal irritation or an inflammation condition optionally selected from or associated with irritable bowel disease, Crohn's disease or chronic diarrhea, or any combination of the foregoing is selected from (a) a DNA virus infection or an RNA virus infection; (b) a mycoplasma infection, a Listeria infection or a Mycobacterium infection; (c) a Streptococcus infection, a Staphylococcus infection, a Vibrio infection, a Salmonella infection, a Shigella infection, an enterotoxigenic, enteropathogenic, enteroinvasive or enterohemorrhagic E. coli infection, a Yersinia infection, a Campylobacter infection, a Pseudomonas infection, a Borrelia infection, a Legionella infection and a Haemophilus infection; (d) pulmonary Aspergillosis, mucosal or oropharyngeal candidiasis and juvenile paracoccidiomycosis; (e) a Candida infection and a Cryptococcus infection; (f) systemic lupus erythematosus, arthritis and diabetes; (g) a solid or a disseminated cancer selected from ovarian cancer, cervical cancer, breast cancer, prostate cancer, liver cancer or carcinoma, a glioma, a lymphoma, a leukemia and a colon cancer; (h) benign prostatic hyperplasia and recurrent condylomata acuminata; (i) adriamycin treatment, cisplatin treatment, mitomycin C treatment, amphotericin B treatment; (j) a γ -radiation therapy; (k) nucleoside analog treatment for viral infection or cancer; (l) surgical wounds and accidental wounds; (m) cyclosporin treatment and corticosteroid treatment; (n) an inflammation condition optionally selected from or associated with asthma, irritable bowel disease, Crohn's disease, chronic diarrhea; or (o) any combination of (a) through (n).

39. The method of claim 38 wherein the subject is a mammal.

40. The method of claim 38 wherein the DNA virus infection or the RNA virus infection is selected from a HSV, CMV, HBV, HCV, HIV, SHIV, SIV, FIV, EBV, HSV-1, HSV-2, HSV-6, HHV-6, HHV-8, adeno-associated virus, measles virus, poxvirus, Poliovirus, human rhinovirus, human papilloma virus and animal papilloma virus infection.

41. The method of claim 40 wherein the subject is a human having an HIV-1 or HIV-2 infection, wherein the subject's CD4 cell count is about 25 to about 100 CD4⁺ cells/mm³.

42. The method of claim 41 wherein the subject is suffering from one or more complications or co-infections associated with AIDS.

43. The method of claim 40 wherein the subject has a pathogen infection or a malignancy and the pathogen or the malignancy does not become resistant to the formula 1 compound over a time normally associated with the development of measurable resistance to any traditional pathogen or malignancy chemotherapy or radiation therapy in at least about 30% of the subjects who are treated with a chemotherapy or a radiation therapy for the pathogen infection or the malignancy other than a formula 1 compound.

44. A method to prevent or treat an immune dysregulation or deficiency condition in a subject in need thereof comprising administering to the subject, or delivering to the subject's tissues, an effective amount of a compound of formula 1 ##STR98## wherein, R¹-R¹⁰ have the

R⁹ are not --CH₂--, or one of R¹-R⁶ and R¹⁰ is an amino acid.

45. The method of claim 44 wherein R⁷ is --O--, --NH-- or --S--.

46. The method of claim 44 wherein R⁸ is --O--, --NH-- or --S--.

47. The method of claim 44 wherein R⁹ is --O--, --NH--, .dbd.N-- or --S--.

48. The method of claim 44 wherein two of R⁷, R⁸ and R⁹ are not --CH₂--.

49. The method of claim 48 wherein R⁷ is --O-- and R⁸ is --O--, R⁷ is --O-- and R⁹ is --O--, R⁸ is --O-- and R⁹ is --O--, R⁷ is --O-- and R⁸ is --N--, R⁷ is --O-- and R⁹ is --NH-- or .dbd.N--, R⁸ is --O-- and R⁹ is --NH-- or .dbd.N--, R⁷ is --O-- and R⁸ is --S--, R⁷ is --O-- and R⁹ is --S--, R⁸ is --O-- and R⁹ is --S--, R⁷ is --NH-- and R⁸ is --NH--, R⁷ is --NH-- and R⁹ is --NH-- or .dbd.N--, R⁸ is --NH-- and R⁹ is --NH-- or .dbd.N--, R⁷ is --NH-- and R⁸ is --O--, R⁷ is --NH-- and R⁹ is --O--, R⁸ is --NH-- and R⁹ is --O--, R⁷ is --NH-- and R⁸ is --S--, R⁷ is --NH-- and R⁹ is --S--, R⁸ is --NH-- and R⁹ is --S--, R⁷ is --S-- and R⁸ is --S--, R⁷ is --S-- and R⁹ is --S--, R⁸ is --S-- and R⁹ is --S--, R⁷ is --S-- and R⁸ is --N--, R⁷ is --S-- and R⁹ is --NH-- or .dbd.N--, R⁸ is --S-- and R⁹ is --NH-- or .dbd.N--, R⁷ is --S-- and R⁸ is --O--, R⁷ is --S-- and R⁹ is --O--, or R⁸ is --S-- and R⁹ is --O--.

50. The method of claim 44 wherein none of R⁷, R⁸ and R⁹ are --CH₂--.

51. The method of claim 44 wherein the immune dysregulation or deficiency condition is associated with an infection, a cancer or a precancer, or the immune dysregulation or deficiency condition is an inflammation condition, an autoimmune disease or a neurodegenerative disorder.

52. The method of claim 1 wherein the subject has a pathogen infection, a cancer or a precancer and subject's antibody response to one or more of the pathogen's antigens or one or more of the cancer's or precancer's antigens is enhanced.

53. 16 α -Bromo-3 β -hydroxy-5 α -androstan-17-one hemihydrate substantially free of other forms of 16 α -bromo-3 β -hydroxy-5 α -androstan-17-one.

54. The 16 α -bromo-3 β -hydroxy-5 α -androstan-17-one hemihydrate substantially free of other forms of 16 α -bromo-3 β -hydroxy-5 α -androstan-17-one of claim 53 wherein 16 α -bromo-3 β -hydroxy-5 α -androstan-17-one hemihydrate comprises at least about 55% w/w of the 16 α -bromo-3 β -hydroxy-5 α -androstan-17-one that is present.

L5 ANSWER 63 OF 112 USPTAFULL on STN

2003:57393 Functional protein expression for rapid cell-free phenotyping.

McCarthy, Laurence, Great Falls, VA, UNITED STATES

Kong, Lilly, Covina, CA, UNITED STATES

Shao, Tang, Rosemead, CA, UNITED STATES

Su, Xin, Irvine, CA, UNITED STATES

US 2003039957 A1 20030227

APPLICATION: US 2001-996187 A1 20011127 (9)

PRIORITY: US 2000-253150P 20001127 (60)

US 2001-297686P 20010612 (60)

US 2001-304533P 20010709 (60)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Disclosed herein are methods for assaying the phenotype of a bioactive molecule in the presence and absence of compounds that are known inhibitors of the phenotypable activity of the bioactive molecule. Also disclosed are methods for discovering compounds that can inhibit the phenotypable activity of a bioactive molecule. The methods and assays of the present invention are useful in developing and monitoring a chemotherapy regimen for a patient, to detect or prevent the emergence of a drug resistant phenotype.

CLM What is claimed is:

1. A method for producing and evaluating a bioactive molecule comprising

bioactive molecule; b) expressing the bioactive molecule encoded by the nucleic acid sequence obtained in step (a), wherein the expressed bioactive molecule has a detectable phenotype; c) contacting the bioactive molecule obtained in step (b) with a compound; and d) detecting the phenotype of the bioactive molecule in the presence or absence of the compound contacted in step (c).

2. The method of claim 1, wherein the bioactive molecule is selected from the group consisting of: a viral molecule, a bacterial molecule, a fungal molecule, a protozoal molecule, a human molecule and an animal molecule.

3. The method of claim 1, wherein the bioactive molecule is a protein further comprising a retrovirus protein, a herpesvirus protein, a hantavirus protein, a hepatitis virus protein, an influenza protein, a myxovirus protein, a picomavirus protein, an adenovirus protein, a poxvirus protein, a flavivirus protein or a coronavirus protein.

4. The method of claim 1, wherein the bioactive molecule is a protein further comprising a streptococcus protein, a staphylococcus protein, an enterococcus protein, a neisseria protein, a salmonella protein, a mycobacteria protein, a bacillus protein, a mycoplasma protein, a chlamydia protein, a francisella protein, a pasteurilla protein, a brucella protein, a pseudomonas protein, a listeria protein, a clostridium protein, a yersinia protein, a vibrio protein, a shigella protein, or an enterobacteriaceae protein.

5. The method of claim 1, wherein the bioactive molecule is a protein further comprising a plasmodium protein, a trypanosome protein, or a cryptosporidium protein.

6. The method of claim 1, wherein the bioactive molecule is a protein further comprising a candida protein, a cryptococcus protein, a malassezia protein, a histoplasma protein, a coccidioides protein, a hyphomyces protein, a blastomyces protein, an aspergillus protein, a penicillium protein, a pseudallescheria protein, a fusarium protein, a paecilomyces protein, a mucor/rhizopus protein, a pneumocystis protein, a rhinosporidium protein, a sporothrix protein, a trichophyton protein, a microsporum protein, an epidermophyton protein, a basidiobolus protein, a conidiobolus protein, a rhizopus protein, a Cunninghamella protein, a paracoccidioides protein, a pseudallescheria protein, or a rhinosporidium protein.

7. The method of claim 1, wherein the nucleic acid sequence encoding the biomolecule further comprises deoxyribonucleic acid or ribonucleic acid.

8. The method of claim 1 or claim 7, wherein the nucleic acid sequence encoding a bioactive molecule further comprises transfer RNA or polyA+ RNA.

9. The method of claim 1, wherein the bioactive molecule further comprises a protein, a glycoprotein, a polysaccharide, a mucopolysaccharide, a lipopolysaccharide, a lipoprotein, a carbohydrate, or a nucleic acid.

10. The method of claim 1, wherein the bioactive molecule encoded by the nucleic acid is expressed in a cell-free eukaryotic cell lysate translation system.

11. The method of claim 1, wherein the bioactive molecule encoded by the nucleic acid is expressed in a cell-free prokaryotic cell lysate translation system.

12. The method of claim 10, wherein the bioactive molecule encoded by the amplified nucleic acid sequence is expressed in a cell-free reticulocyte lysate translation system.

13. The method of claim 12, wherein the bioactive molecule encoded by the amplified nucleic acid sequence is expressed in a cell-free reticulocyte lysate coupled transcription/translation system.

14. The method of claim 13, wherein the bioactive molecule encoded by the nucleic acid sequence and expressed in a cell-free reticulocyte lysate coupled transcription/translation system is a nucleic acid selected from the group consisting of: deoxyribonucleic acid, ribonucleic acid, polyA+ RNA, tRNA, and rRNA.

15. The method of claim 1, wherein the nucleic acid sequence that encodes the bioactive molecule further comprises a second nucleic acid sequence operably linked to said bioactive molecule.

16. The method of claim 15, wherein the second nucleic acid sequence

17. The method of claim 15, wherein the second nucleic acid sequence comprises a purification motif.

18. The method of claim 15, wherein the second nucleic acid sequence encodes a gene product or fragment thereof comprising a purification motif.

19. The method of claim 1, wherein the bioactive molecule is contacted with a compound selected from the group consisting of: an anti-viral compound, an anti-bacterial compound, an anti-fungal compound, an anti-cancer compound, an immunosuppressive compound, a hormone, a cytokine, a lymphokine, a chemokine, an enzyme, a polypeptide, a polynucleotide, and a nucleoside analogue.

20. The method of claim 1, wherein detecting the phenotype of the bioactive molecule further comprises assaying the enzymatic activity of the bioactive molecule.

21. The method of claim 20, wherein assaying the enzymatic activity of the bioactive molecule further comprises assaying the bio active molecule for a resistance phenotype to the compound.

22. The method of claim 1, wherein detecting the phenotype of the bioactive molecule further comprises assaying the affinity of the bioactive molecule for the compound.

23. The method of claim 22, wherein assaying the affinity of the bioactive molecule for the compound further comprises assaying the bioactive molecule for a resistance phenotype to the compound.

24. The method of claim 1, wherein detecting the phenotype of the bioactive molecule further comprises assaying the structure of the bioactive molecule.

25. The method of claim 24, wherein assaying the structure of the bioactive molecule comprises predicting a resistance phenotype to the compound.

26. The method of claim 1, wherein the method is preceeded by the step of: amplifying a nucleic acid sequence in a cell-free system, wherein the nucleic acid sequence comprises a bioactive molecule.

27. The method of claim 1, wherein the nucleic acid encoding a bioactive molecule is amplified by a reaction selected from the group consisting of: a **polymerase chain reaction**, a ligase chain reaction, a transcription mediated amplification reaction, a nucleic acid sequence based amplification reaction, and a strand displacement amplification reaction.

28. The method of claim 1, wherein amplifying the nucleic acid encoding the biomolecule comprises a **polymerase chain reaction** further comprising one or more nested **primer** sets.

29. The method of claim 1, wherein amplifying the nucleic acid encoding the biomolecule NO:2, SEQ ID NO:3, SEQ ID NO:4.

30. The method of claim 1 or claim 26, wherein the method is preceeded by the step of: extracting one or more specemins from a patient afflicted with a disease state, wherein the specemins comprise a bioactive molecule associated with a disease state.

31. A method for producing and evaluating a bioactive molecule comprising the steps of: a) isolating at least one organism or tissue, wherein the organism or tissue comprises a bioactive molecule associated with a disease state; b) amplifying a nucleic acid sequence in a cell-free system, wherein the nucleic acid sequence comprises the bioactive molecule and is obtained from the organism or tissue isolated in step (a); c) expressing the bioactive molecule encoded by the nucleic acid sequence obtained in step (b), wherein the expressed bioactive molecule has a detectable phenotype further comprising resistance to a first compound; d) contacting the bioactive molecule obtained in step (c) with a second compound; and e) detecting the phenotype of the bioactive molecule in the presence or absence of the second compound contacted in step (d).

32. The method of claim 1, wherein the method is preceeded by the step of: amplifying a nucleic acid sequence in a cell-free system, wherein the nucleic acid sequence comprises a bioactive molecule.

33. The method of claim 1 or claim 26, wherein the method is preceeded by the step of: extracting one or more specemins from a patient

bioactive molecule associated with the disease state.

34. A method for producing and evaluating a bioactive molecule comprising the steps of: a) isolating at least one organism or tissue, wherein the organism or tissue comprises a bioactive molecule associated with a disease state; b) amplifying a nucleic acid sequence in a cell-free system, wherein the nucleic acid sequence comprises the bioactive molecule and is obtained from the organism or tissue isolated in step (a); c) expressing the bioactive molecule encoded by the nucleic acid sequence obtained in step (b), wherein the expressed bioactive molecule has a detectable phenotype further comprising resistance to a first compound; d) contacting the bioactive molecule obtained in step (c) with a second compound; and e) detecting the phenotype of the bioactive molecule in the presence or absence of the second compound contacted in step (d).

L5 ANSWER 64 OF 112 USPATFULL on STN

2003:52389 Novel nucleic acid sequences encoding a human ubiquitin protease, lipase, dynamin, short chain dehydrogenase, and ADAM-TS metalloprotease and uses therefor.

Glucksmann, Maria Alexandra, Lexington, MA, UNITED STATES

Kapeller-Libermann, Rosana, Chestnut Hill, MA, UNITED STATES

Meyers, Rachel E., Newton, MA, UNITED STATES

Rudolph-Owen, Laura A., Jamaica Plain, MA, UNITED STATES

Millennium Pharmaceuticals, Inc. (U.S. corporation)

US 2003037350 A1 20030220

APPLICATION: US 2002-163547 A1 20020605 (10)

PRIORITY: US 2000-185503P 20000228 (60)

US 2000-182009P 20000211 (60)

US 2000-182408P 20000214 (60)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention provides isolated nucleic acids molecules that encode novel polypeptides. The invention also provides antisense nucleic acid molecules, recombinant expression vectors containing the nucleic acid molecules of the invention, host cells into which the expression vectors have been introduced, and nonhuman transgenic animals in which a sequence of the invention has been introduced or disrupted. The invention still further provides isolated proteins, fusion proteins, antigenic peptides and antibodies. Diagnostic methods utilizing compositions of the invention are also provided.

CLM What is claimed is:

1. An isolated nucleic acid molecule selected from the group consisting of: a) a nucleic acid molecule comprising a nucleotide sequence which is at least 60% identical to the nucleotide sequence of SEQ ID NO:2, 4, 6, 8, 12, 14, 15, 17, or 22 or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Numbers PTA-1870, PTA-2014, PTA-2200, or PTA-2010; b) a nucleic acid molecule comprising a fragment of at least 300 nucleotides of the nucleotide sequence of SEQ ID NO:2, 4, 6, 8, 12, 14, 15, 17, or 22 or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Numbers PTA-1870, PTA-2014, PTA-2200, or PTA-2010; c) a nucleic acid molecule which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:1, 3, 7, 13, 16, 21, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Numbers PTA-1870, PTA-2014, PTA-2200, or PTA-2010; d) a nucleic acid molecule which encodes a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:1, 3, 7, 13, 16, 21, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Numbers PTA-1870, PTA-2014, PTA-2200, or PTA-2010, wherein the fragment comprises at least 15 contiguous amino acids of SEQ ID NO:1, 3, 7, 13, 16, 21, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Numbers PTA-1870, PTA-2014, PTA-2200, or PTA-2010; and e) a nucleic acid molecule which encodes a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:1, 3, 7, 13, 16, 21, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Numbers PTA-1870, PTA-2014, PTA-2200, or PTA-2010, wherein the nucleic acid molecule hybridizes to a nucleic acid molecule comprising SEQ ID NO:2, 4, 6, 8, 12, 14, 15, 17, or 22, or a complement thereof, under stringent conditions.

2. The isolated nucleic acid molecule of claim 1, which is selected from the group consisting of: a) a nucleic acid comprising the nucleotide sequence of SEQ ID NO:2, 4, 6, 8, 12, 14, 15, 17, or 22 or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Numbers PTA-1870, PTA-2014, PTA-2200, or PTA-2010; and b) a nucleic acid molecule which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:1, 3, 7, 13, 16, 21, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Numbers PTA-1870, PTA-2014, PTA-2200, or PTA-2010.

3. The nucleic acid molecule of claim 1 further comprising vector nucleic acid sequences.
4. The nucleic acid molecule of claim 1 further comprising nucleic acid sequences encoding a heterologous polypeptide.
5. A host cell which contains the nucleic acid molecule of claim 1.
6. The host cell of claim 5 which is a mammalian host cell.
7. A non-human mammalian host cell containing the nucleic acid molecule of claim 1.
8. An isolated polypeptide selected from the group consisting of: a) a polypeptide which is encoded by a nucleic acid molecule comprising a nucleotide sequence which is at least 60% identical to a nucleic acid comprising the nucleotide sequence of SEQ ID NO:2, 4, 6, 8, 12, 14, 15, 17, or 22 or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Numbers PTA-1870, PTA-2014, PTA-2200, or PTA-2010, or a complement thereof; b) a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:1, 3, 7, 13, 16, 21, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Numbers PTA-1870, PTA-2014, PTA-2200, or PTA-2010, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a nucleic acid molecule comprising SEQ ID NO:2, 4, 6, 8, 12, 14, 15, 17, or 22, or a complement thereof under stringent conditions; and c) a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:1, 3, 7, 13, 16, 21, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Numbers PTA-1870, PTA-2014, PTA-2200, or PTA-2010, wherein the fragment comprises at least 15 contiguous amino acids of SEQ ID NO:1, 3, 7, 13, 16, 21.
9. The isolated polypeptide of claim 8 comprising the amino acid sequence of SEQ ID NO:1, 3, 7, 13, 16, 21.
10. The polypeptide of claim 8 further comprising heterologous amino acid sequences.
11. An antibody which selectively binds to a polypeptide of claim 8.
12. A method for producing a polypeptide selected from the group consisting of: a) a polypeptide comprising the amino acid sequence of SEQ ID NO:1, 3, 7, 13, 16, 21, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Numbers PTA-1870, PTA-2014, PTA-2200, or PTA-2010; b) a polypeptide comprising a fragment of the amino acid sequence of SEQ ID NO:1, 3, 7, 13, 16, 21, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Numbers PTA-1870, PTA-2014, PTA-2200, or PTA-2010, wherein the fragment comprises at least 15 contiguous amino acids of SEQ ID NO:1, 3, 7, 13, 16, 21, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Numbers PTA-1870, PTA-2014, PTA-2200, or PTA-2010; and c) a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:1, 3, 7, 13, 16, 21, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Numbers PTA-1870, PTA-2014, PTA-2200, or PTA-2010, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a nucleic acid molecule comprising SEQ ID NO:2, 4, 6, 8, 12, 14, 15, 17, or 22; comprising culturing the host cell of claim 5 under conditions in which the nucleic acid molecule is expressed.
13. A method for detecting the presence of a polypeptide of claim 8 in a sample, comprising: a) contacting the sample with a compound which selectively binds to a polypeptide of claim 8; and b) determining whether the compound binds to the polypeptide in the sample.
14. The method of claim 13, wherein the compound which binds to the polypeptide is an antibody.
15. A kit comprising a compound which selectively binds to a polypeptide of claim 8 and instructions for use.
16. A method for detecting the presence of a nucleic acid molecule of claim 1 in a sample, comprising the steps of: a) contacting the sample with a nucleic acid probe or **primer** which selectively hybridizes to the nucleic acid molecule; and b) determining whether the nucleic acid probe or **primer** binds to a nucleic acid molecule in the sample.
17. The method of claim 16, wherein the sample comprises mRNA molecules and is contacted with a nucleic acid probe.

18. A kit comprising a compound which selectively hybridizes to a nucleic acid molecule of claim 1 and instructions for use.

19. A method for identifying a compound which binds to a polypeptide of claim 8 comprising the steps of: a) contacting a polypeptide, or a cell expressing a polypeptide of claim 8 with a test compound; and b) determining whether the polypeptide binds to the test compound.

20. The method of claim 19, wherein the binding of the test compound to the polypeptide is detected by a method selected from the group consisting of: a) detection of binding by direct detecting of test compound/polypeptide binding; and, b) detection of binding using a competition binding assay.

21. A method for modulating the activity of a polypeptide of claim 8 comprising contacting a polypeptide or a cell expressing a polypeptide of claim 8 with a compound which binds to the polypeptide in a sufficient concentration to modulate the activity of the polypeptide.

22. A method for identifying a compound which modulates the activity of a polypeptide of claim 8, comprising: a) contacting a polypeptide of claim 8 with a test compound; and b) determining the effect of the test compound on the activity of the polypeptide to thereby identify a compound which modulates the activity of the polypeptide.

L5 ANSWER 65 OF 112 USPTAFULL on STN

2003:46308 Transgenic animals and cell lines for screening drugs effective for the treatment or prevention of Alzheimer's disease.

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US 2003033621 A1 20030213

APPLICATION: US 2001-964667 A1 20010928 (9)

PRIORITY: US 1997-38908P 19970226 (60)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Disclosed are transgenic animals and transfected cell lines expressing a protein associated with Alzheimer's Disease, neuroectodermal tumors, malignant astrocytomas, and glioblastomas. Also disclosed is the use of such transgenic animals and transfected cell lines to screen potential drug candidates for treating or preventing Alzheimer's disease, neuroectodermal tumors, malignant astrocytomas, and glioblastomas. The invention also relates to new antisense oligonucleotides, ribozymes, triplex forming DNA and external guide sequences that can be used to treat or prevent Alzheimer's disease, neuroectodermal tumors, malignant astrocytomas, and glioblastomas.

CLM What is claimed is:

1. A DNA construct, which comprises a DNA molecule of Seq. ID No. 1 or a DNA molecule which is at least 40% homologous thereto, or a fragment thereof, wherein said DNA molecule is under control of a heterologous neuro-specific promoter.

2. The DNA construct of claim 1, which is contained within a vector.

3. The DNA construct of claim 1, which is contained by a viron.

4. The DNA construct of claim 1, wherein said DNA molecule has Seq. ID No. 1.

5. A host cell transformed with the DNA construct of claim 1.

6. The host cell line of claim 5, which is a neuronal cell.

7. A transgenic non-human animal, all of whose germ and somatic cells comprises the DNA molecule of Seq. ID No. 1 or a DNA molecule which is at least 40% homologous thereto.

8. The transgenic non-human animal of claim 7, wherein the DNA molecule contained in each germ and somatic cell has Seq. ID No. 1.

9. The transgenic non-human animal of claim 7, wherein the protein coded for by said DNA molecule is overexpressed in the brain of the animal.

10. An in vitro method for screening a candidate drug that is potentially useful for the treatment or prevention of Alzheimer's disease, neuroectodermal tumors, malignant astrocytomas, and glioblastomas, which comprises (a) contacting a candidate drug with the host cell line of claim 5, and (b) detecting at least one of the following: (i) the suppression or prevention of expression of the protein coded for by the DNA construct; (ii) the increased degradation of the protein coded for by the DNA construct; or (iii) the reduction of frequency of at least one of neuritic sprouting, nerve cell death,

neurites and axons in the host; due to the drug candidate compared to a control cell line which has not contacted the candidate drug.

11. The method of claim 10, wherein said protein has Seq. ID No. 2.

12. The method of claim 10, wherein said protein is over-expressed by said host cell.

13. The method of claim 10, wherein said cell is a neuronal cell.

14. An in vivo method for screening a candidate drug that is potentially useful for the treatment or prevention of Alzheimer's disease, neuroectodermal tumors, malignant astrocytomas, and glioblastomas, which comprises (a) administering a candidate drug to the transgenic animal of claim 7, and (b) detecting at least one of the following: (i) the suppression or prevention of expression of the protein coded for by the DNA construct contained by said animal; (ii) the increased degradation of the protein coded for by the DNA construct contained by said animal; or (iii) the reduction of frequency of at least one of neuritic sprouting, nerve cell death, degenerating neurons, neurofibrillary tangles, or irregular swollen neurites and axons in the host; due to the drug candidate compared to a control animal which has not received the candidate drug.

15. The method of claim 14, wherein the DNA construct contained by said animal has Seq. ID No. 1.

16. The method of claim 14, wherein the protein coded for by the DNA construct contained by said animal is over-expressed in the brain of said animal.

17. An antisense **oligonucleotide** which is complementary to an NTP mRNA sequence corresponding to nucleotides 150-1139 of Seq. ID No. 1.

18. The antisense **oligonucleotide** of claim 17, which is a 15 to 40-mer.

19. The antisense **oligonucleotide** of claim 17, wherein said antisense **oligonucleotide** is selected from the group consisting of Seq ID Nos. 9 to 11.

20. The antisense **oligonucleotide** of claim 17, which is deoxyribonucleic acid.

21. The antisense **oligonucleotide** of claim 17, which is a deoxyribonucleic acid phosphorothioate.

22. The antisense **oligonucleotide** of claim 17, which is a derivative of a deoxyribonucleic acid or a deoxyribonucleic acid phosphorothioate.

23. A pharmaceutical composition comprising the antisense **oligonucleotide** of claim 17 and a pharmaceutically acceptable carrier.

24. A ribozyme comprising a target sequence which is complementary to an NTP mRNA sequence corresponding to nucleotides 150-1139 of Seq. ID No. 1.

25. A pharmaceutical composition comprising the ribozyme of claim 24 and a pharmaceutically acceptable carrier.

26. An oligodeoxynucleotide that forms triple stranded regions with the a region of AD7c-NTP coding nucleic acid and having the sequence 3'X5'-L-5'X3', wherein X comprises an AD7c-NTP nucleic acid sequence corresponding to nucleotides 150-1139 of Seq. ID No. 1, and wherein L represents an **oligonucleotide** linker or a bond.

27. A pharmaceutical composition comprising the oligodeoxynucleotide of claim 26 and a pharmaceutically acceptable carrier.

28. An oligodeoxynucleotide that forms triple stranded regions with the a region of AD7c-NTP coding nucleic acid and having the sequence 5'X3'-L-3'X5', wherein X comprises an AD7c-NTP nucleic acid sequence corresponding to nucleotides 150-1139 of Seq. ID No. 1, and wherein L represents an **oligonucleotide** linker or a bond.

29. A pharmaceutical composition comprising the oligodeoxynucleotide of claim 28 and a pharmaceutically acceptable carrier.

30. A ribonucleotide external guide nucleic acid molecule, comprising, a 10-mer nucleotide sequence corresponding to nucleotides 150-1139 of Seq. ID No. 1 fused to a 3'NCCA nucleotide sequence, wherein N is a purine.

31. The ribonucleotide external guide nucleic acid molecule of claim 30

32. A pharmaceutical composition comprising the ribonucleotide of claim 30 and a pharmaceutically acceptable carrier.

33. A method for to treat or prevent dementias of the Alzheimer's type of neuronal degeneration; or to treat or prevent neuroectodermal tumors, malignant astrocytomas, or glioblastomas, comprising administering to an animal in need thereof an antisense **oligonucleotide**, a ribozyme, a triple helix-forming **oligonucleotide** or an ribonucleotide external guide sequence of any one of claims 17, 24, 26, 28, or 30.

34. The method of claim 32, wherein said antisense **oligonucleotide**, ribozyme, triple helix-forming **oligonucleotide** or ribonucleotide external guide sequence is administered to said animal as part of a pharmaceutically acceptable carrier.

L5 ANSWER 66 OF 112 USPTAFULL on STN

2003:37603 Human cDNAs and proteins and uses thereof.

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Tanaka, Hiroaki, Antony, FRANCE

GENSET, S.A., Paris, FRANCE, 75008 (non-U.S. corporation)

US 2003027248 A1 20030206

APPLICATION: US 2001-924340 A1 20010806 (9)

PRIORITY: US 2001-305456P 20010713 (60)

US 2001-302277P 20010629 (60)

US 2001-298698P 20010615 (60)

US 2001-293574P 20010525 (60)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention concerns GENSET polynucleotides and polypeptides. Such GENSET products may be used as reagents in forensic analyses, as chromosome markers, as tissue/cell/organelle-specific markers, in the production of expression vectors. In addition, they may be used in screening and diagnosis assays for abnormal GENSET expression and/or biological activity and for screening compounds that may be used in the treatment of GENSET-related disorders.

CLM What is claimed is:

1. An isolated polynucleotide, comprising a nucleic acid sequence selected from the group consisting of: a) a polynucleotide of an even SEQ ID NO., or of a human cDNA of a deposited clone, encoding at least any single integer from 6 to 500 amino acids of any one odd SEQ ID NO., b) a polynucleotide of an even SEQ ID NO., or of a human cDNA of a deposited clone, encoding the signal peptide sequence of any one odd SEQ ID NO., c) a polynucleotide of an even SEQ ID NO., or of a human cDNA of a deposited clone, encoding a mature polypeptide sequence of any one odd SEQ ID NO., d) a polynucleotide of an even SEQ ID NO., or of a human cDNA of a deposited clone, encoding a full length polypeptide sequence of any one odd SEQ ID NO., e) a polynucleotide of an even SEQ ID NO., or of a human cDNA of a deposited clone, encoding a polypeptide sequence of a biologically active fragment of any one odd SEQ ID NO., f) a polynucleotide encoding a polypeptide sequence of at least any single integer from 6 to 500 amino acids of any one odd SEQ ID NO. or of a polypeptide encoded by a human cDNA of a deposited clone, g) a polynucleotide encoding a polypeptide sequence of a signal peptide of any one odd SEQ ID NO. or of a signal peptide encoded by a human cDNA of a deposited clone, h) a polynucleotide encoding a polypeptide sequence of a mature polypeptide of any one odd SEQ ID NO. or of a mature polypeptide encoded by a human cDNA of a deposited clone, i) a polynucleotide encoding a polypeptide sequence of a full length polypeptide of any one odd SEQ ID NO. or of a mature polypeptide encoded by a human cDNA of a deposited clone, j) a polynucleotide encoding a polypeptide sequence of a biologically polypeptide of any one odd SEQ ID NO., or of a biologically polypeptide encoded by a human cDNA of a deposited clone, k) a polynucleotide of any one of a) through j) further comprising an expression vector, l) a host cell recombinant for a polynucleotide of a) through k) above, m) a non-human transgenic animal comprising the host cell of k), n) a polynucleotide of a) through j) further comprising a physiologically acceptable carrier.

2. A polypeptide comprising an amino acid sequence selected from the group consisting of: a) any single integer from 6 to 500 amino acids of any one odd SEQ ID NO. or of a polypeptide encoded by a human cDNA of a deposited clone; b) a signal peptide sequence of any one odd SEQ ID NO. or encoded by a human cDNA of a deposited clone; c) a mature polypeptide sequence of any one odd SEQ ID NO. or encoded by a human cDNA of a deposited clone; d) a full length polypeptide sequence of any one odd SEQ ID NO. or encoded by a human cDNA of a deposited clone; e) a polypeptide of a) through d) further comprising a physiologically acceptable carrier.

providing a population of host cells comprising the polynucleotide of claim 1; b) culturing said population of host cells under conditions conducive to the production of a polypeptide of claim 2 within said host cells; and c) purifying said polypeptide from said population of host cells.

4. A method of making a polypeptide, said method comprising: a) providing a population of cells comprising a polynucleotide encoding the polypeptide of claim 2, operably linked to a promoter; b) culturing said population of cells under conditions conducive to the production of said polypeptide within said cells; and c) purifying said polypeptide from said population of cells.

5. An antibody that specifically binds to the polypeptide of claim 2.

6. A method of binding a polypeptide of claim 2 to an antibody of claim 5, comprising contacting said antibody with said polypeptide under conditions in which antibody can specifically bind to said polypeptide.

7. A method of determining whether a GENSET gene is expressed within a mammal, said method comprising the steps of: a) providing a biological sample from said mammal b) contacting said biological sample with either of: i) a polynucleotide that hybridizes under stringent conditions to the polynucleotide of claim 1; or ii) a polypeptide that specifically binds to the polypeptide of claim 2; and c) detecting the presence or absence of hybridization between said polynucleotide and an RNA species within said sample, or the presence or absence of binding of said polypeptide to a protein within said sample; wherein a detection of said hybridization or of said binding indicates that said GENSET gene is expressed within said mammal.

8. The method of claim 7, wherein said polynucleotide is a **primer**, and wherein said hybridization is detected by detecting the presence of an amplification product comprising the sequence of said **primer**.

9. The method of claim 7, wherein said polypeptide is an antibody.

10. A method of determining whether a mammal has an elevated or reduced level of GENSET gene expression, said method comprising the steps of: a) providing a biological sample from said mammal; and b) comparing the amount of the polypeptide of claim 2, or of an RNA species encoding said polypeptide, within said biological sample with a level detected in or expected from a control sample; wherein an increased amount of said polypeptide or said RNA species within said biological sample compared to said level detected in or expected from said control sample indicates that said mammal has an elevated level of said GENSET gene expression, and wherein a decreased amount of said polypeptide or said RNA species within said biological sample compared to said level detected in or expected from said control sample indicates that said mammal has a reduced level of said GENSET gene expression.

11. A method of identifying a candidate modulator of a GENSET polypeptide, said method comprising: a) contacting the polypeptide of claim 2 with a test compound; and b) determining whether said compound specifically binds to said polypeptide; wherein a detection that said compound specifically binds to said polypeptide indicates that said compound is a candidate modulator of said GENSET polypeptide.

12. The method of claim 11, further comprising testing the biological activity of said GENSET polypeptide in the presence of said candidate modulator, wherein an alteration in the biological activity of said GENSET polypeptide in the presence of said compound in comparison to the activity in the absence of said compound indicates that the compound is a modulator of said GENSET polypeptide.

13. A method for the production of a pharmaceutical composition comprising a) identifying a modulator of a GENSET polypeptide using the method of claim 11; and b) combining said modulator with a physiologically acceptable carrier.

L5 ANSWER 67 OF 112 USPATFULL on STN

2003:37516 Human cDNAs and proteins and uses thereof.

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GENSET, S.A., Paris, FRANCE, 75008 (non-U.S. corporation)

US 2003027161 A1 20030206

APPLICATION: US 2001-992600 A1 20011113 (9)

PRIORITY: WO 2001-IB1715 20010806

US 2001-305456P 20010713 (60)

US 2001-302277P 20010629 (60)

US 2001-298698P 20010615 (60)

AB The invention concerns GENSET polynucleotides and polypeptides. Such GENSET products may be used as reagents in forensic analyses, as chromosome markers, as tissue/cell/organelle-specific markers, in the production of expression vectors. In addition, they may be used in screening and diagnosis assays for abnormal GENSET expression and/or biological activity and for screening compounds that may be used in the treatment of GENSET-related disorders.

CLM What is claimed is:

1. An isolated polynucleotide, comprising a nucleic acid sequence selected from the group consisting of: a) a polynucleotide of SEQ ID NO:3, or of a human cDNA of deposited clone 181-40-4-0-A11-F, encoding at least any single integer from 6 to 500 amino acids of SEQ ID NO:4; b) a polynucleotide of SEQ ID NO:3, or of a human cDNA of deposited clone 18140-4-0-A11-F, encoding the signal peptide sequence of SEQ ID NO:4; c) a polynucleotide of SEQ ID NO:3, or of a human cDNA of deposited clone 181-40-4-0-A11-F, encoding a mature polypeptide sequence of SEQ ID NO:4; d) a polynucleotide of SEQ ID NO:3, or of a human cDNA of deposited clone 181-40-4-0-A11-F, encoding a full length polypeptide sequence of SEQ ID NO:4; e) a polynucleotide of SEQ ID NO:3, or of a human cDNA of deposited clone 181-40-4-0-A11-F, encoding a polypeptide sequence of a biologically active fragment of SEQ ID NO:4; f) a polynucleotide encoding a polypeptide sequence of at least any single integer from 6 to 500 amino acids of SEQ ID NO:4 or of a polypeptide encoded by a human cDNA of deposited clone 181-40-4-0-A11-F; g) a polynucleotide encoding a polypeptide sequence of a signal peptide of SEQ ID NO:4 or of a signal peptide encoded by a human cDNA of deposited clone 181-40-4-0-A11-F; h) a polynucleotide encoding a polypeptide sequence of a mature polypeptide of SEQ ID NO:4 or of a mature polypeptide encoded by a human cDNA of deposited clone 181-404-0-A11-F; i) a polynucleotide encoding a polypeptide sequence of a full length polypeptide of SEQ ID NO:4 or of a mature polypeptide encoded by a human cDNA of deposited clone 181-404-0-A11-F; j) a polynucleotide encoding a polypeptide sequence of a biologically active polypeptide of SEQ ID NO:4, or of a biologically active polypeptide encoded by a human cDNA of deposited clone 181-40-4-0-A11-F; k) a polynucleotide of any one of a) through j) further comprising an expression vector; l) a host cell recombinant for a polynucleotide of a) through k) above; m) a non-human transgenic animal comprising the host cell of k); and n) a polynucleotide of a) through j) further comprising a physiologically acceptable carrier.

2. A polypeptide comprising an amino acid sequence selected from the group consisting of: a) any single integer from 6 to 500 amino acids of SEQ ID NO:4 or of a polypeptide encoded by a human cDNA of deposited clone 181-40-4-0-A11-F; b) a signal peptide sequence of SEQ ID NO:4 or encoded by a human cDNA of deposited clone 181-404-0-A11-F; c) a mature polypeptide sequence of SEQ ID NO:4 or encoded by a human cDNA of deposited clone 181-404-0-A11-F; d) a full length polypeptide sequence of SEQ ID NO:4 or encoded by a human cDNA of deposited clone 181-40-4-0-A11-F; and e) a polypeptide of a) through d) further comprising a physiologically acceptable carrier.

3. A method of making a polypeptide, said method comprising: a) providing a population of host cells comprising the polynucleotide of claim 1; b) culturing said population of host cells under conditions conducive to the production of a polypeptide of claim 2 within said host cells; and c) purifying said polypeptide from said population of host cells.

4. A method of making a polypeptide, said method comprising: a) providing a population of cells comprising a polynucleotide encoding the polypeptide of claim 2, operably linked to a promoter; b) culturing said population of cells under conditions conducive to the production of said polypeptide within said cells; and c) purifying said polypeptide from said population of cells.

5. An antibody that specifically binds to the polypeptide of claim 2.

6. A method of binding a polypeptide of claim 2 to an antibody of claim 5, comprising contacting said antibody with said polypeptide under conditions in which antibody can specifically bind to said polypeptide.

7. A method of determining whether a SCPx gene is expressed within a mammal, said method comprising the steps of: a) providing a biological sample from said mammal; b) contacting said biological sample with either of: i) a polynucleotide that hybridizes under stringent conditions to the polynucleotide of claim 1; or ii) a polypeptide that specifically binds to the polypeptide of claim 2; and c) detecting the presence or absence of hybridization between said polynucleotide and an RNA species within said sample, or the presence or absence of binding of

of said hybridization or of said binding indicates that said SCPHx gene is expressed within said mammal.

8. The method of claim 7, wherein said polynucleotide is a **primer**, and wherein said hybridization is detected by detecting the presence of an amplification product comprising the sequence of said **primer**.

9. The method of claim 7, wherein said polypeptide is an antibody.

10. A method of determining whether a mammal has an elevated or reduced level of a SCPHx gene expression, said method comprising the steps of: a) providing a biological sample from said mammal; and b) comparing the amount of the polypeptide of claim 2, or of an RNA species encoding said polypeptide, within said biological sample with a level detected in or expected from a control sample; wherein an increased amount of said polypeptide or said RNA species within said biological sample compared to said level detected in or expected from said control sample indicates that said mammal has an elevated level of said SCPHx gene expression, and wherein a decreased amount of said polypeptide or said RNA species within said biological sample compared to said level detected in or expected from said control sample indicates that said mammal has a reduced level of said SCPHx gene expression.

11. A method of identifying a candidate modulator of a SCPHx polypeptide, said method comprising: a) contacting the polypeptide of claim 2 with a test compound; and b) determining whether said compound specifically binds to said polypeptide; wherein a detection that said compound specifically binds to said polypeptide indicates that said compound is a candidate modulator of said SCPHx polypeptide.

12. The method of claim 11, further comprising testing the biological activity of said SCPHx polypeptide in the presence of said candidate modulator, wherein an alteration in the biological activity of said SCPHx polypeptide in the presence of said compound in comparison to the activity in the absence of said compound indicates that the compound is a modulator of said SCPHx polypeptide.

13. A method for the production of a pharmaceutical composition comprising a) identifying a modulator of a SCPHx polypeptide using the method of claim 11; and b) combining said modulator with a physiologically acceptable carrier.

L5 ANSWER 68 OF 112 USPTAFULL on STN

2003:37490 Method for rapid detection and identification of bioagents.

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US 2003027135 A1 20030206

APPLICATION: US 2001-798007 A1 20010302 (9)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Method for detecting and identifying unknown bioagents, including bacteria, viruses and the like, by a combination of nucleic acid amplification and molecular weight determination using primers which hybridize to conserved sequence regions of nucleic acids derived from a bioagent and which bracket variable sequence regions that uniquely identify the bioagent. The result is a "base composition signature" (BCS) which is then matched against a database of base composition signatures, by which the bioagent is identified.

CLM What is claimed is:

1. A method of identifying an unknown bioagent comprising: (a) contacting nucleic acid from said bioagent with at least one pair of **oligonucleotide primers** which hybridize to sequences of said nucleic acid, wherein said sequences flank a variable nucleic acid sequence of the bioagent; (b) amplifying said variable nucleic acid sequence to produce an amplification product; (c) determining the molecular mass of said amplification product; and (d) comparing said molecular mass to one or more molecular masses of amplification products obtained by performing steps (a)-(c) on a plurality of known organisms, wherein a match identifies said unknown bioagent.

2. The method of claim 1, wherein said sequences to which said at least one pair of **oligonucleotide primers** hybridize are highly conserved.

3. The method of claim 1, wherein said amplifying step comprises **polymerase chain reaction**.

4. The method of claim 1, wherein said amplifying step comprises ligase chain reaction or strand displacement amplification.

5. The method of claim 1, wherein said bioagent is a bacterium, virus, cell or spore.
6. The method of claim 1, wherein said nucleic acid is ribosomal RNA.
7. The method of claim 1, wherein said nucleic acid encodes RNase P or an RNA-dependent RNA polymerase.
8. The method of claim 1, wherein said amplification product is ionized prior to molecular mass determination.
9. The method of claim 1, further comprising the step of isolating nucleic acid from said bioagent prior to contacting said nucleic acid with said at least one pair of **oligonucleotide primers**.
10. The method of claim 1, further comprising the step of performing steps (a)-(d) using a different **oligonucleotide primer** pair and comparing the results to one or more molecular mass amplification product obtained by performing steps (a)-(c) on a different plurality of known organisms from those in step (d).
11. The method of claim 1, wherein said one or more molecular masses are contained in a database of molecular masses.
12. The method of claim 1, wherein said amplification product is ionized by electrospray ionization, matrix-assisted laser desorption or fast atom bombardment.
13. The method of claim 1, wherein said molecular mass is determined by mass spectrometry.
14. The method of claim 11, wherein said mass spectrometry is selected from the group consisting of Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS), ion trap, quadrupole, magnetic sector, time of flight (TOF), Q-TOF and triple quadrupole.
15. The method of claim 1, further comprising performing step (b) in the presence of an analog of adenine, thymidine, guanosine or cytidine having a different molecular weight than adenosine, thymidine, guanosine or cytidine.
16. The method of claim 1, wherein said **oligonucleotide primer** comprises a base analog at positions 1 and 2 of each triplet within said **primer**, wherein said base analog binds with increased affinity to its complement compared to the native base.
17. The method of claim 16, wherein said **primer** comprises a universal base at position 3 of each triplet within said **primer**.
18. The method of claim 16, wherein said base analog is selected from the group consisting of 2,6-diaminopurine, propyne T, propyne G, phenoxazines and G-clamp.
19. The method of claim 16, wherein said universal base is selected from the group consisting of inosine, guanidine uridine, 5-nitroindole, 3-nitropyrrole, dP, dK, and 1-(2-deoxy- β -D-ribofuranosyl)-imidazole-4-carboxamide.
20. A method of identifying an unknown bioagent comprising: contacting nucleic acid from said bioagent with at least one pair of **oligonucleotide primers** which hybridize to sequences of said nucleic acid, wherein said sequences flank a variable nucleic acid sequence; amplifying said variable nucleic acid sequence to produce an amplification product; determining the base composition of said amplification product; and comparing said base composition to one or more base compositions of amplification products obtained by performing steps (a)-(c) on a plurality of known organisms, wherein a match identifies said unknown bioagent.
21. The method of claim 20, wherein said sequences to which said at least one pair of **oligonucleotide primers** hybridize are highly conserved.
22. The method of claim 20, wherein said amplifying step comprises **polymerase chain reaction**.
23. The method of claim 20, wherein said amplifying step comprises ligase chain reaction or strand displacement amplification.
24. The method of claim 20, wherein said bioagent is a bacterium, virus, cell or spore.

26. The method of claim 20, wherein said nucleic acid encodes RNase P or an RNA-dependent RNA polymerase.

27. The method of claim 20, wherein said amplification product is ionized prior to base composition determination.

28. The method of claim 20, further comprising the step of isolating nucleic acid from said bioagent prior to contacting said nucleic acid with said at least one pair of **oligonucleotide primers**.

29. The method of claim 20, further comprising the step of performing steps (a)-(d) using a different **oligonucleotide primer** pair and comparing the results to one or more base compositions of amplification product obtained by performing steps (a)-(c) on a different plurality of known organisms from those in step (d).

30. The method of claim 20, wherein said one or more base composition signatures are contained in a database of base composition signatures.

31. The method of claim 20, wherein said amplification product is ionized by electrospray ionization, matrix-assisted laser desorption or fast atom bombardment.

32. The method of claim 20, wherein said base composition signature is determined by mass spectrometry.

33. The method of claim 32, wherein said mass spectrometry is selected from the group consisting of Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS), ion trap, quadrupole, magnetic sector, time of flight (TOF), q-TOF and triple quadrupole.

34. The method of claim 20, further comprising performing step (b) in the presence of an analog of adenine, thymidine, guanosine or cytidine having a different molecular weight than adenosine, thymidine, guanosine or cytidine.

35. The method of claim 20, wherein said **oligonucleotide primer** comprises a base analog at positions 1 and 2 of each triplet within said **primer**, wherein said base analog binds with increased affinity to its complement compared to the native base.

36. The method of claim 35, wherein said **primer** comprises a universal base at position 3 of each triplet within said **primer**.

37. The method of claim 35, wherein said base analog is selected from the group consisting of 2,6-diaminopurine, propyne T, propyne G, phenoxazines and G-clamp.

38. The method of claim 36, wherein said universal base is selected from the group consisting of inosine, guanidine uridine, 5-nitroindole, 3-nitropyrrole, dP, dK, and 1-(2-deoxy- β -D-ribofuranosyl)-imidazole-4-carboxamide.

39. A method for detecting a single nucleotide polymorphism in an individual, comprising the steps of: isolating nucleic acid from said individual; contacting said nucleic acid with **oligonucleotide primers** which hybridize to regions of said nucleic acid which flank a region comprising said potential polymorphism; amplifying said region to produce an amplification product; determining the molecular mass of said amplification product; comparing said molecular mass to the molecular mass of said region in an individual known to have said polymorphism, wherein if said molecular masses are the same then said individual has said polymorphism.

40. The method of claim 39, wherein said polymorphism is associated with a disease.

41. The method of claim 39, wherein said polymorphism is a blood group antigen.

42. The method of claim 39, wherein said amplification step is the **polymerase chain reaction**.

43. The method of claim 39, wherein said amplification step is ligase chain reaction or strand displacement amplification.

44. The method of claim 39, wherein said amplification product is ionized prior to mass determination.

45. The method of claim 39, wherein said amplification product is ionized by electrospray ionization, matrix-assisted laser desorption or

46. The method of claim 39, wherein said **primers** hybridize to conserved sequences.

47. The method of claim 39, wherein said molecular mass is determined by mass spectrometry.

48. The method of claim 47, wherein said mass spectrometry is selected from the group consisting of Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS), ion trap, quadrupole, magnetic sector, time of flight (TOF), Q-TOF and triple quadrupole.

L5 ANSWER 69 OF 112 USPTAFULL on STN

2003:23313 Single chain monoclonal antibody fusion reagents that regulate transcription in vivo.

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US 2003017149 A1 20030123

APPLICATION: US 2001-939769 A1 20010828 (9)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method of screening a DNA construct library for a single chain monoclonal antibody s fusion reagent capable of binding a transcriptional associated biomolecule in vivo is described. Single chain monoclonal antibody fusion reagents capable of binding transcriptional associated biomolecules in vivo are provided. Single chain monoclonal antibody fusion reagents which are capable of regulating transcription in vivo are also provided. Therapeutic methods for regulating the transcription of a gene in vivo are also described. A method is further provided for screening a plurality of compounds for specific binding affinity with a single chain monoclonal antibody fusion reagent. A method is also described for diagnosing a physiological disorder manifested by an abnormal level of a transcription associated biomolecule. A DNA construct (pVP16Zeo) as well as primers for the construction and screening of single chain monoclonal antibody fusion reagent libraries to facilitate the isolation and production of single chain monoclonal antibody fusion reagents in yeast and E.coli are also provided. A kit for screening a DNA construct library for a single chain monoclonal antibody fusion reagent capable of binding a transcriptional associated biomolecule in vivo is also provided.

CLM What is claimed is:

1. A method of screening a DNA construct library for a single chain monoclonal antibody fusion reagent capable of binding a transcriptional associated biomolecule in vivo comprising: cloning a nucleic acid fragment which encodes a peptide DBD of a transcription factor into an expression vector to yield a construct (1) such that the DBD may be expressed in a bio-active form and bind a corresponding DNA regulatory sequence binding site in a heterologous host cell, fusing a nucleic acid fragment which encodes an antigenic portion of a transcriptional associated biomolecule into construct 1, in the same translation reading frame of the nucleic acid fragment which encodes the DBD of a transcription factor, to yield a construct (2), cloning an sFv library into a DNA construct to yield a construct (3) such that a single chain monoclonal antibody may be expressed in bio-active form and bind a corresponding antigen in a heterologous host cell, fusing a nucleic acid fragment which encodes a trans-activation peptide into construct 3, in the same translation reading frame of the nucleic acid fragment which encodes the single chain monoclonal antibody, to yield a construct (4) such that a resulting chimeric sFv/trans-activation peptide may be expressed in bio-active form and bind the corresponding antigen in a heterologous host cell, providing a heterologous host cell harboring a detectable gene under transcriptional control of the DNA regulatory sequence binding site corresponding to the DBD encoded by construct 2, introducing constructs 2 and 4 into the heterologous host cell harboring a detectable gene under transcriptional control of the DNA regulatory sequence binding site corresponding to the DBD encoded by construct 2, such that both constructs may be expressed, and identifying a DNA construct 4 which encodes a single chain monoclonal antibody reagent capable of binding the transcriptional associated biomolecule in vivo by selecting for expression of the detectable gene.

2. The method of claim 1 further comprising fusing at least one nucleic acid fragment which encodes an intracellular targeting signal in the same translation reading frame to the nucleic acid fragment which encodes the single chain monoclonal antibody in construct 4, to yield a modified construct (5) such that a resulting single chain monoclonal antibody fusion reagent may be expressed in bio-active form and bind the corresponding antigen in a heterologous host cell.

3. The method of claim 1 further comprising fusing at least one nucleic acid fragment which encodes an intracellular targeting signal in the

encodes the single chain monoclonal antibody in construct 4, and deleting the TA, to yield a modified construct (6) such that a resulting single chain monoclonal antibody fusion reagent may be expressed in bio-active form and bind the corresponding antigen in a heterologous host cell.

4. The method of claim 2 wherein the transcriptional associated biomolecule is selected from the group consisting essentially of a transcription factor, ligand, hormone, nuclear hormone receptor, DNA binding domain of nuclear hormone receptor, tumor associated protein, protein kinase, protein phosphatase, GTP binding protein, adaptor protein, secondary messenger of an intracellular signaling molecule, and a protein derived from an etiological agent.

5. The method of claim 4 wherein the transcriptional associated biomolecule is selected from the group consisting of Ras, Grb2, phospholipase C γ -PLC γ , phosphatidylinositol 3-kinase-PI3K, Syp, mitogen activated protein kinase-MAPK, jun kinase-JNK, androgen receptor (AR), thyroid hormone receptor (TR), glucocorticoid receptor (GR), ATF-1, ATF-2, ATF-3, ATF-4, ATF-6, CREB and CREM.

6. The method of claim 3 wherein the transcriptional associated biomolecule is selected from the group consisting essentially of a transcription factor, ligand, hormone, nuclear hormone receptor, DNA binding domain of nuclear hormone receptor, tumor associated protein, protein kinase, protein phosphatase, GTP binding protein, adaptor protein, secondary messenger of an intracellular signaling molecule, and a protein derived from an etiological agent.

7. The method of claim 6 wherein the transcriptional associated biomolecule is selected from the group consisting of Ras, Grb2, phospholipase C γ -PLC γ , phosphatidylinositol 3-kinase-PI3K, Syp, mitogen activated protein kinase-MAPK, jun kinase-JNK, androgen receptor (AR), thyroid hormone receptor (TR), glucocorticoid receptor (GR), ATF-1, ATF-2, ATF-3, ATF-4, ATF-6, CREB and CREM.

8. A method of screening a DNA construct library for a single chain monoclonal antibody fusion reagent capable of binding a transcriptional associated biomolecule in vivo comprising: providing an expression construct (1) which encodes a peptide DBD of a transcription factor and comprises a cloning site for fusing a nucleic acid fragment which encodes an antigenic portion of a transcriptional associated biomolecule in the same translation reading frame of the nucleic acid fragment which encodes the DBD of a transcription factor, to yield a construct (2), providing a DNA construct (3) which encodes a trans-activation peptide and comprises a cloning site for fusing an sFv library in the same translation reading frame of the trans-activation peptide, to yield a construct (4) such that a resulting chimeric sFv/trans-activation peptide may be expressed in bio-active form and bind a transcriptional associated biomolecule in a heterologous host cell, providing a heterologous host cell, harboring a detectable gene under transcriptional control of the DNA regulatory sequence binding site corresponding to the DBD encoded by construct 2, for introducing constructs 2 and 4 into the heterologous host cell, such that both constructs may be expressed, and identifying a DNA construct 4 which encodes a single chain monoclonal antibody reagent capable of binding the transcriptional associated biomolecule in vivo by selecting for expression of the detectable gene.

9. A single chain monoclonal antibody fusion reagent capable of binding a transcriptional associated biomolecule in vivo isolated by a method comprising: cloning a nucleic acid fragment which encodes a peptide DBD of a transcription factor into an expression vector to yield a construct (1) such that the DBD may be expressed in a bio-active form and bind a corresponding DNA regulatory sequence binding site in a heterologous host cell, fusing a nucleic acid fragment which encodes an antigenic portion of a transcriptional associated biomolecule into construct 1, in the same translation reading frame of the nucleic acid fragment which encodes the DBD of a transcription factor, to yield a construct (2), cloning an sFv library into a DNA construct to yield a construct (3) such that a single chain monoclonal antibody may be expressed in bio-active form and bind a corresponding antigen in a heterologous host cell, fusing a nucleic acid fragment which encodes a trans-activation peptide into construct 3, in the same translation reading frame of the nucleic acid fragment which encodes the single chain monoclonal antibody, to yield a construct (4) such that a resulting chimeric sFv/trans-activation peptide may be expressed in bio-active form and bind the corresponding antigen in a heterologous host cell, providing a heterologous host cell harboring a detectable gene under transcriptional control of the DNA regulatory sequence binding site corresponding to the DBD encoded by construct 2, introducing constructs 2 and 4 into the

control of the DNA regulatory sequence binding site corresponding to the DBD encoded by construct 2, such that both constructs may be expressed, identifying a DNA construct 4 which encodes a single chain monoclonal antibody reagent capable of binding the transcriptional associated biomolecule in vivo by selecting for expression of the detectable gene, and isolating the single chain monoclonal antibody fusion reagent capable of binding the transcriptional associated biomolecule in vivo.

10. The single chain monoclonal antibody fusion reagent of claim 9 further comprising fusing at least one nucleic acid fragment which encodes an intracellular targeting signal in the same translation reading frame to the nucleic acid fragment which encodes the single chain monoclonal antibody in construct 4, to yield a modified construct (5) such that a resulting single chain monoclonal antibody fusion reagent may be expressed in bio-active form and bind the corresponding antigen in a heterologous host cell.

11. The single chain monoclonal antibody fusion reagent of claim 9 further comprising fusing at least one nucleic acid fragment which encodes an intracellular targeting signal in the same translation reading frame to the nucleic acid fragment which encodes the single chain monoclonal antibody in construct 4, and deleting the TA, to yield a modified construct (6) such that a resulting single chain monoclonal antibody fusion reagent may be expressed in bio-active form and bind the corresponding antigen in a heterologous host cell.

12. A single chain monoclonal antibody fusion reagent according to claim 9 which is capable of regulating transcription in vivo.

13. A single chain monoclonal antibody fusion reagent according to claim 10 which is capable of regulating transcription in vivo.

14. A single chain monoclonal antibody fusion reagent according to claim 11 which is capable of regulating transcription in vivo.

15. A therapeutic method for regulating the transcription of a gene in vivo comprising administering an effective amount of a single chain monoclonal antibody fusion reagent capable of binding a transcriptional associated biomolecule in vivo identified by a method comprising: providing an expression construct (1) which encodes a peptide DBD of a transcription factor and comprises a cloning site for fusing a nucleic acid fragment which encodes an antigenic portion of a transcriptional associated biomolecule in the same translation reading frame of the nucleic acid fragment which encodes the DBD of a transcription factor, to yield a construct (2), providing a DNA construct (3) which encodes a trans-activation peptide and comprises a cloning site for fusing an sFv library in the same translation reading frame of the trans-activation peptide, to yield a construct (4) such that a resulting chimeric sFv/trans-activation peptide may be expressed in bio-active form and bind a transcriptional associated biomolecule in a heterologous host cell, providing a heterologous host cell, harboring a detectable gene under transcriptional control of the DNA regulatory sequence binding site corresponding to the DBD encoded by construct 2, for introducing constructs 2 and 4 into the heterologous host cell, such that both constructs may be expressed, and identifying a DNA construct 4 which encodes a single chain monoclonal antibody reagent capable of binding the transcriptional associated biomolecule in vivo by selecting for expression of the detectable gene.

16. A therapeutic method for regulating the transcription of a gene in vivo according to claim 15 wherein the single chain monoclonal antibody fusion reagent capable of binding a transcriptional associated biomolecule in vivo comprises at least one intracellular targeting signal fused to the single chain monoclonal antibody.

17. A method of screening a plurality of compounds for specific binding affinity with a single chain monoclonal antibody fusion reagent capable of binding a transcriptional associated biomolecule in vivo identified by a method comprising: providing an expression construct (1) which encodes a peptide DBD of a transcription factor and comprises a cloning site for fusing a nucleic acid fragment which encodes an antigenic portion of a transcriptional associated biomolecule in the same translation reading frame of the nucleic acid fragment which encodes the DBD of a transcription factor, to yield a construct (2), providing a DNA construct (3) which encodes a trans-activation peptide and comprises a cloning site for fusing an sFv library in the same translation reading frame of the trans-activation peptide, to yield a construct (4) such that a resulting chimeric sFv/trans-activation peptide may be expressed in bio-active form and bind a transcriptional associated biomolecule in a heterologous host cell, providing a heterologous host cell, harboring a detectable gene under transcriptional control of the DNA regulatory sequence binding site corresponding to the DBD encoded by construct 2,

that both constructs may be expressed, and identifying a DNA construct 4 which encodes a single chain monoclonal antibody reagent capable of binding the transcriptional associated biomolecule in vivo by selecting for expression of the detectable gene, and screening a plurality of compounds comprising the steps of: providing a plurality of compounds, combining the single chain monoclonal antibody fusion reagent with each of a plurality of compounds for a time sufficient to allow binding under suitable conditions; and detecting binding of said single chain monoclonal antibody fusion reagent to each of the plurality of compounds, thereby identifying the compounds which specifically bind said single chain monoclonal antibody fusion reagent.

18. A method for diagnosing a physiological disorder manifested by abnormal levels of a transcription associated biomolecule, said method comprising: contacting a biological sample with a labelled single chain monoclonal antibody fusion reagent or a portion thereof according to claim 9 whereby said antibody reagent binds to said transcription associated biomolecule to form a complex, separating unbound labelled antibody reagent from said complex, measuring the amount of bound labelled antibody reagent in said complex; and, comparing the quantity of labelled antibody reagent in said biological sample to the quantity of labelled antibody reagent which binds to normal biological samples under identical conditions.

19. A pVP16Zeo library expression vector (ATCC deposit #_____) for the construction and screening of single chain monoclonal antibody fusion reagent libraries, comprising zeocin selection to facilitate the isolation and production of single chain monoclonal antibody fusion reagents in yeast and E. coli.

20. A kit for screening a DNA construct library for a single chain monoclonal antibody fusion reagent capable of binding a transcriptional associated biomolecule in vivo; comprising in a container: an expression construct (1) which encodes a peptide DBD of a transcription factor and comprises a cloning site for fusing a nucleic acid fragment which encodes an antigenic portion of a transcriptional associated biomolecule in the same translation reading frame of the nucleic acid fragment which encodes the DBD of a transcription factor, to yield a construct (2), and a DNA construct (3) which encodes a trans-activation peptide and comprises a cloning site for fusing an sFv library in the same translation reading frame of the trans-activation peptide, to yield a construct (4) such that a resulting chimeric sFv/trans-activation peptide may be expressed in bio-active form and bind a transcriptional associated biomolecule in a heterologous host cell, and a heterologous host cell harboring a detectable gene under transcriptional control of the DNA regulatory sequence binding site corresponding to the DBD encoded by construct 2, for introducing constructs 2 and 4 into the heterologous host cell, such that both constructs may be expressed, and a means for identifying a DNA construct 4 which encodes a single chain monoclonal antibody reagent capable of binding the transcriptional associated biomolecule in vivo by selecting for expression of the detectable gene.

21. A kit for screening a DNA construct library for a single chain monoclonal antibody fusion reagent capable of binding a transcriptional associated biomolecule in vivo according to claim 20 wherein DNA construct 3 is pVP16Zeo (ATCC deposit #_____) .

22. A kit for screening a DNA construct library for a single chain monoclonal antibody fusion reagent capable of binding a transcriptional associated biomolecule in vivo according to claim 21 wherein **primers** are provided for human sFv library construction.

23. A kit for screening a DNA construct library for a single chain monoclonal antibody fusion reagent capable of binding a transcriptional associated biomolecule in vivo according to claim 22 wherein **primers** select e d from the group consisting essentially of (SEQ ID NOs: 3-8. are provided for human sFv library construction.

L5 ANSWER 70 OF 112 USPTAFULL on STN

2002:322456 Quantitative determination of nucleic acid amplification products.

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US 2002182620 A1 20021205

APPLICATION: US 2002-43415 A1 20020110 (10)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to a method for detecting the amount of a target polynucleotide in a sample. A combination is provided in a medium. The combination comprises (i) a sample suspected of containing the target polynucleotide, the target polynucleotide being in single

that is common with a sequence of the target polynucleotide, and (iii) a predetermined amount of an oligonucleotide probe that has a sequence that hybridizes with the sequence that is common. The combination is subjected to conditions for amplifying the target polynucleotide and the reference polynucleotide. The conditions permit formation of substantially non-dissociative complexes of the target polynucleotide and the reference polynucleotide, respectively, with the oligonucleotide probe. Furthermore, the predetermined amount of the oligonucleotide probe is less than the expected amount of the amplified target polynucleotide. The ratio of the amount of the complex of the target polynucleotide with the oligonucleotide probe to the amount of the complex of the reference polynucleotide with the oligonucleotide probe is determined. Determination of the ratio is facilitated by employing second and third oligonucleotide probes. The second oligonucleotide probe has a sequence that hybridizes only with the second sequence of the target polynucleotide. The third oligonucleotide probe has a sequence that hybridizes only with a respective second sequence of the reference polynucleotide. The ratio is related to the known amount of the reference polynucleotide to determine the amount of the target polynucleotide in the sample. One or more reference polynucleotides may be employed with a corresponding third oligonucleotide probe for each reference probe. Kits for carrying out the above methods are also disclosed. The method is particularly applicable to the amplification and detection of RNA.

CLM

What is claimed is:

1. A method for detecting the amount of a target polynucleotide in a sample, said method comprising: (a) providing in combination in a medium (i) a sample suspected of containing said target polynucleotide, said target polynucleotide being in single stranded form, (ii) a reference polynucleotide comprising a sequence that is common with a sequence of said target polynucleotide, and (iii) a predetermined amount of an **oligonucleotide** probe that has a sequence that hybridizes with said sequence that is common; (b) subjecting said combination to conditions for amplifying said target polynucleotide and said reference polynucleotide wherein said conditions permit formation of substantially non-dissociative complexes of said target polynucleotide and said reference polynucleotide, respectively, with said **oligonucleotide** probe and wherein said predetermined amount of said **oligonucleotide** probe is less than the expected amount of said amplified target polynucleotide, (c) determining the ratio of the amount of said complex of said target polynucleotide with said **oligonucleotide** probe to the amount of said complex of said reference polynucleotide with said **oligonucleotide** probe, and (d) relating said ratio to said known amount of said reference polynucleotide to determine the amount of said target polynucleotide in said sample.

2. The method of claim 1 wherein a signal is produced by the complex of said target polynucleotide with said **oligonucleotide** probe wherein said signal is different from a signal produced by the complex of said reference polynucleotide with said **oligonucleotide** probe and the ratio of said signals is related to the amount of said reference polynucleotide to determine the amount of said target polynucleotide in said sample.

3. The method of claim 1 wherein said amplification is selected from the group consisting of NASBA, 3SR, SDA and amplifications utilizing Q β -replicase.

4. The method of claim 1 wherein said polynucleotide is DNA.

5. The method of claim 1 wherein said polynucleotide is RNA.

6. A method for detecting the amount of a target polynucleotide in a sample, said method comprising: (a) providing in combination in a medium (i) a sample suspected of containing said target polynucleotide, said target polynucleotide being in single stranded form, (ii) predetermined amounts of one or more reference polynucleotides, each of said reference polynucleotides comprising a first sequence that is common with a first sequence of said target polynucleotide and a second sequence that is different from a second sequence of said target polynucleotide, (iii) a predetermined amount of a first **oligonucleotide** probe that has a sequence that hybridizes with said sequence that is common, (iv) a second **oligonucleotide** probe that has a sequence that hybridizes only with said second sequence of said target polynucleotide, and (v) one or more third **oligonucleotide** probes, each of said third **oligonucleotide** probes having a sequence that hybridizes only with a respective second sequence of one of said reference polynucleotides; (b) subjecting said combination to isothermal conditions for amplifying with equal efficiency said target polynucleotide and said one or more reference polynucleotides wherein said conditions permit formation of a substantially non-dissociative first termolecular complex of said target polynucleotide, said first

substantially non-dissociative second termolecular complex of each of said reference polynucleotide with said first **oligonucleotide** probe and a respective third **oligonucleotide** probe and wherein said predetermined amount of said first **oligonucleotide** probe is less than the expected amount of said amplified target polynucleotide, (c) determining the ratio of the amount of said first termolecular complex to the amount of each of said second termolecular complexes, and (d) relating each of said ratios to the predetermined amount of each of said reference polynucleotides to determine the amount of said target polynucleotide in said sample.

7. The method of claim 6 wherein said first termolecular complex has a first signal producing system and each of said second termolecular complexes has a second signal producing system wherein a signal produced by said first signal producing system is different from a signal produced by each of said second signal producing systems.

8. The method of claim 7 wherein the ratio of said signals is determined and related to the predetermined amount of said reference polynucleotide to determine the amount of said target polynucleotide in said sample.

9. The method of claim 7 wherein said signal producing systems comprise labels selected from the group consisting of a luminescent energy donor and acceptor pair, a singlet oxygen generator and chemiluminescent reactant pair, and an enzyme pair wherein a product of the first enzyme serves as a substrate for the second enzyme.

10. The method of claim 6 wherein said amplification is selected from the group consisting of NASBA, 3SR, SDA and amplifications utilizing Q β -replicase.

11. The method of claim 6 wherein said polynucleotide is DNA.

12. The method of claim 6 wherein said polynucleotide is RNA.

13. The method of claim 6 wherein one of said first and second **oligonucleotide** probes is labeled with a sensitizer.

14. The method of claim 6 wherein one of said first and second **oligonucleotide** probes is labeled with a chemiluminescent compound.

15. The method of claim 6 wherein said predetermined amount of said first **oligonucleotide** probe is about 50-fold less than the expected amount of said amplified target polynucleotide.

16. A method for detecting the amount of a target polynucleotide in a sample, said method comprising: (a) providing in combination in a medium (i) a sample suspected of containing said target polynucleotide, said target polynucleotide being in single stranded form, (ii) predetermined amounts of one or more reference polynucleotides, each of said reference polynucleotides comprising a first sequence that is common with a first sequence of said target polynucleotide and a second sequence that is different from a second sequence of said target polynucleotide, (iii) a predetermined amount of a first **oligonucleotide** probe that has a sequence that hybridizes with said sequence that is common wherein said first **oligonucleotide** probe has, or is capable of having, a sensitizer attached thereto, (iv) a second **oligonucleotide** probe that has a sequence that hybridizes only with said second sequence of said target polynucleotide wherein said second **oligonucleotide** probe has, or is capable of having, a first chemiluminescent compound attached thereto, and (v) one or more third **oligonucleotide** probes, each having a sequence that hybridizes only with a respective second sequence of one of said reference polynucleotide wherein each of said third **oligonucleotide** probes has, or is capable of having, a second chemiluminescent compound attached thereto, said first and said second chemiluminescent compounds differ in signal produced when activated by said photosensitizer; said second chemiluminescent compound being different for each of said third **oligonucleotide** probes, (b) subjecting said combination to isothermal conditions for amplifying with equal efficiency said target polynucleotide and each of said reference polynucleotide wherein said conditions permit formation of a substantially non-dissociative first termolecular complex of said target polynucleotide, said first **oligonucleotide** probe and said second **oligonucleotide** probe and a substantially non-dissociative second termolecular complex of each of said reference polynucleotides with said first **oligonucleotide** probe and a respective third **oligonucleotide** probe and wherein said predetermined amount of said first **oligonucleotide** probe is less than the expected amount of said amplified target polynucleotide, (c) determining the ratio of the amount of said signal produced by said first termolecular complex to the amount of signal produced by each of said second termolecular complexes, and (d) relating each of said

determine the amount of said target polynucleotide in said sample.

17. The method of claim 16 wherein said chemiluminescent compounds are each independently selected from the group consisting of enol ethers, enamines, 9-alkylidene-N-alkylacridans, arylvinylethers, dioxenes, arylimidazoles, 9-alkylidene-xanthenes and lucigenin.

18. The method of claim 16 wherein said sensitizer is a photosensitizer.

19. The method of claim 18 wherein said photosensitizer is selected from the group consisting of methylene blue, rose bengal, porphyrins and phthalocyanines.

20. The method of claim 16 wherein said amplification is selected from the group consisting of NASBA, 3SR, SDA and amplifications utilizing Q β -replicase.

21. The method of claim 16 wherein said polynucleotide is DNA.

22. The method of claim 16 wherein said polynucleotide is RNA.

23. A kit for use in the determination of the amount of a target polynucleotide in a sample, said kit comprising in packaged combination: (a) reagents for conducting an amplification of said target polynucleotide, (b) predetermined amounts of one or more reference polynucleotides, each of said reference polynucleotides comprising a first sequence that is common with a first sequence of said target polynucleotide and a second sequence that is different from a second sequence of said target polynucleotide, (c) a predetermined amount of a first **oligonucleotide** probe that has a sequence that hybridizes with said sequence that is common, (d) a second **oligonucleotide** probe that has a sequence that hybridizes only with said second sequence of said target polynucleotide, and (e) one or more third **oligonucleotide** probes, each of said third **oligonucleotide** probes having a sequence that hybridizes only with a respective second sequence of one of said reference polynucleotide;

24. The kit of claim 23 further comprising a signal producing system comprising a label on one of said first or said second or said third **oligonucleotide** probes wherein said label is a particle having a chemiluminescent compound associated therewith.

25. The kit of claim 23 further comprising a signal producing system comprising a label on one of said first **oligonucleotide** probes wherein said label is a sensitizer.

26. The kit of claim 23 wherein said reagents for conducting an amplification comprise a promoter and an enzyme.

27. The kit of claim 26 wherein said amplification is selected from the group consisting of NASBA, 3SR, SDA and amplifications utilizing Q β -replicase.

28. The kit of claim 23 wherein said target polynucleotide is DNA.

29. The kit of claim 23 wherein said target polynucleotide is RNA.

30. A kit for use in a determination of the amount of a specific RNA in a sample, said kit comprising in packaged combination: (a) one or more reference RNA's, (b) a promoter, (c) an enzyme and (d) predetermined amounts of one or more reference polynucleotides, each of said reference polynucleotides comprising a first sequence that is common with a first sequence of said target polynucleotide and a second sequence that is different from a second sequence of said target polynucleotide, (e) a predetermined amount of a first **oligonucleotide** probe that has a sequence that hybridizes with said sequence that is common, said first **oligonucleotide** probe being labeled with a sensitizer, (f) a second **oligonucleotide** probe that has a sequence that hybridizes only with said second sequence of said target polynucleotide, said second **oligonucleotide** probe being labeled with a chemiluminescer, and (g) one or more third **oligonucleotide** probes, each of said third **oligonucleotide** probes having a sequence that hybridizes only with a respective second sequence of one of said reference polynucleotide; each of said third **oligonucleotide** probes being labeled with a chemiluminescer, said chemiluminescer being different from that of said second **oligonucleotide** probe and different for each of said third **oligonucleotide** probes.

31. The kit of claim 30 wherein said sensitizer is a photosensitizer.

32. The kit of claim 30 where said chemiluminescer is a singlet oxygen reactive olefin.

2002:297432 Non-stochastic generation of genetic vaccines.

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US 6479258 B1 20021112

APPLICATION: US 2000-495052 20000131 (9)

PRIORITY: US 1995-8311P 19951207 (60)

DOCUMENT TYPE: Utility; GRANTED.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention provides methods of obtaining vaccines by use of non-stochastic methods of directed evolution (DirectEvolution.TM.). These methods include non-stochastic polynucleotide site-saturation mutagenesis (Gene Site Saturation Mutagenesis.TM.) and non-stochastic polynucleotide reassembly (GeneReassembly.TM.). Through use of the claimed methods, vectors can be obtained which exhibit increased efficacy for use as genetic vaccines. Vectors obtained by using the methods can have, for example, enhanced antigen expression, increased uptake into a cell, increased stability in a cell, ability to tailor an immune response, and the like.

CLM What is claimed is:

1. A method for obtaining an immunomodulatory polynucleotide that has an optimized modulatory effect on an immune response as compared to the response prior to optimization, or encodes a polypeptide that has an optimized modulatory effect on an immune response as compared to the response prior to optimization, the method comprising: creating a library of non-stochastically generated progeny polynucleotides from a parental polynucleotide set, wherein optimization is achieved by at least one directed evolution method in any combination, permutation and iterative manner.

2. The method of claim 1, wherein said optimized modulatory effect on an immune response is induced by a genetic vaccine vector.

3. A method for obtaining an immunomodulatory polynucleotide that has an optimized modulatory effect on an immune response as compared to the response prior to optimization, or encodes a polypeptide that has an optimized modulatory effect on an immune response as compared to the response prior to optimization, the method comprising: screening a library of non-stochastically generated progeny polynucleotides to identify an optimized non-stochastically generated progeny polynucleotide that has, or encodes a polypeptide that has, a modulatory effect on an immune response; wherein the optimized non-stochastically generated polynucleotide or the polypeptide encoded by the non-stochastically generated polynucleotide exhibits an enhanced ability to modulate an immune response compared to a parental polynucleotide from which the library was created.

4. The method of claim 3, wherein said optimized modulatory effect on an immune response is induced by a genetic vaccine vector.

5. A method for obtaining an immunomodulatory polynucleotide that has an optimized modulatory effect on an immune response as compared to the response prior to optimization, or encodes a polypeptide that has an optimized modulatory effect on an immune response as compared to the response prior to optimization, the method comprising: a) creating a library of non-stochastically generated progeny polynucleotides from a parental polynucleotide set; and b) screening the library to identify an optimized non-stochastically generated progeny polynucleotide that has, or encodes a polypeptide that has, a modulatory effect on an immune response induced by a vector; wherein the optimized non-stochastically generated polynucleotide or the polypeptide encoded by the non-stochastically generated polynucleotide exhibits an enhanced ability to modulate an immune response compared to a parental polynucleotide from which the library was created; and whereby optimization is achieved using one or more directed evolution methods in any combination, permutation, and iterative manner.

6. The method of claim 5, wherein said optimized modulatory effect on an immune response is induced by a genetic vaccine vector.

7. The method of any claims 1, 3 or 5, wherein the optimized non-stochastically generated polynucleotide is incorporated into a vector.

8. The method of any of claims 1, 3 or 5, wherein the optimized non-stochastically generated polynucleotide, or a polypeptide encoded by the optimized non-stochastically generated polynucleotide, is administered in conjunction with a vector.

9. The method of any of claims 1, 3 or 5, wherein the library of non-stochastically generated progeny polynucleotides is created by a

saturation mutagenesis, and any combination, permutation and iterative manner.

10. The method of any of claims 1, 3 or 5, wherein the optimized non-stochastically generated polynucleotide that has a modulatory effect on an immune response is obtained by: a) non-stochastically reassembling at least two parental template polynucleotide, each of which is, or encodes a molecule that is, involved in modulating an immune response; wherein the first and second parental templates differ from each other in two or more nucleotides, to produce a library of non-stochastically generated polynucleotides; and b) screening the library to identify at least one optimized non-stochastically generated polynucleotide that exhibits, either by itself or through the encoded molecule, an enhanced ability to modulate an immune response in comparison to a parental polynucleotide from which the library was created.

11. The method of claim 10, wherein the method further comprises the steps of: c) subjecting an optimized non-stochastically generated polynucleotide to a further round of non-stochastic reassembly with at least one additional polynucleotide, which is the same or different from the first and second polynucleotides, to produce a further library of recombinant polynucleotides; d) screening the library produced in c) to identify at least one further optimized non-stochastically generated polynucleotide that exhibits an enhanced ability to modulate an immune response in comparison to a parental polynucleotide from which the library was created; and e) optionally repeating c) and d) as necessary, until a desirable further optimized non-stochastically generated polynucleotide that exhibits an enhanced ability to modulate an immune response than a form of the nucleic acid from which the library was created.

12. The method of any of claims 1, 3 or 5, wherein the optimized non-stochastically generated polynucleotide encodes a polypeptide that interacts with a cellular receptor involved in mediating an immune response; wherein the polypeptide acts as an agonist or antagonist of the receptor.

13. The method of claim 12, wherein the cellular receptor is a macrophage scavenger receptor.

14. The method of claim 12, wherein the cellular receptor is selected from the group consisting of a cytokine receptor and a chemokine receptor.

15. The method of claim 14, wherein the chemokine receptor is CCR5 or CCR6.

16. The method of claim 12, wherein the polypeptide mimics the activity of a natural ligand for the receptor but does not induce immune reactivity to said natural ligand.

17. The method of claim 12, wherein the library is screened by: i) expressing the non-stochastically generated progeny polynucleotides so that the encoded polypeptides are produced as fusions with a protein displayed on the surface of a replicable genetic package; ii) contacting the replicable genetic packages with a plurality of cells that display the receptor; and iii) identifying cells that exhibit a modulation of an immune response mediated by the receptor.

18. The method of claim 17, wherein the replicable genetic package is selected from the group consisting of a bacteriophage, a cell, a spore, and a virus.

19. The method of claim 18, wherein the replicable genetic package is an M13 bacteriophage and the protein is encoded by geneIII or geneVIII.

20. The method of claim 12, which method further comprises introducing the optimized non-stochastically generated polynucleotide into a genetic vaccine vector and administering the vector to a mammal, wherein the peptide or polypeptide is expressed and acts as an agonist or antagonist of the receptor.

21. The method of claim 12, which method further comprises producing the polypeptide encoded by the optimized non-stochastically generated polynucleotide and introducing the polypeptide into a mammal in conjunction with a genetic vaccine vector.

22. The method of claim 12, wherein the optimized non-stochastically generated polynucleotide is inserted into an antigen-encoding nucleotide sequence of a genetic vaccine vector.

23. The method of claim 22, wherein the optimized non-stochastically

encodes an M-loop of an HBsAg polypeptide.

24. The method of any of claims 1, 3 or 5, wherein the optimized non-stochastically generated polynucleotide comprises a nucleotide sequence rich in unmethylated CpG.

25. The method of any of claims 1, 3 or 5, wherein the optimized non-stochastically generated polynucleotide encodes a polypeptide that inhibits an allergic reaction.

26. The method of claim 25, wherein the polypeptide is selected from the group consisting of interferon- α , interferon- γ , IL-10, IL-12, an antagonist of IL-4, an antagonist of IL-5, and an antagonist of IL-13.

27. The method of 1, wherein the optimized recombinant polynucleotide encodes an antagonist of IL-10.

28. The method of claim 27, wherein the antagonist of IL-10 is soluble or defective IL-10 receptor or IL-20/MDA-7.

29. The method of any of claims 1-6, wherein the optimized non-stochastically generated polynucleotide encodes a co-stimulator.

30. The method of claim 29, wherein the co-stimulator is B7-1 (CD80) or B7-2 (CD86) and the screening step involves selecting variants with altered activity through CD28 or CTLA-4.

31. The method of claim 29, wherein the co-stimulator is CD1, CD40, CD154 (ligand for CD40) or CD150 (SLAM).

32. The method of claim 29, wherein the co-stimulator is a cytokine.

33. The method of claim 32, wherein the cytokine is selected from the group consisting of IL-1, IL-2, IL-3, IL4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, GM-CSF, G-CSF, TNF- α , IFN- α , IFN- γ , and IL-20 (MDA-7).

34. The method of 33, wherein the library of non-stochastically generated polynucleotides is screened by testing the ability of cytokines encoded by the non-stochastically generated polynucleotides to activate cells which contain a receptor for the cytokine.

35. The method of claim 34, wherein the cells contain a heterologous nucleic acid that encodes the receptor for the cytokine.

36. The method of 33, wherein the cytokine is interleukin-12 and the screening is performed by: growing mammalian cells which contain the genetic vaccine vector in a culture medium; and detecting whether T cell proliferation or T cell differentiation is induced by contact with the culture medium.

37. The method of 33, wherein the cytokine is interferon- α and the screening is performed by: i) expressing the non-stochastically generated polynucleotides so that the encoded polypeptides are produced as fusions with a protein displayed on the surface of a replicable genetic package; ii) contacting the replicable genetic packages with a plurality of B cells; and iii) identifying phage library members that are capable of inhibiting proliferation of the B cells.

38. The method of claim 33, wherein the immune response of interest is differentiation of T cells to T_H1 cells and the screening is performed by contacting a population of T cells with the cytokines encoded by the members of the library of recombinant polynucleotides and identifying library members that encode a cytokine that induces the T cells to produce IL-2 and interferon- γ .

39. The method of claim 32, wherein the cytokine encoded by the optimized non-stochastically generated polynucleotide exhibits reduced immunogenicity compared to a cytokine encoded by a non-optimized polynucleotide, and the reduced immunogenicity is detected by introducing a cytokine encoded by the non-stochastically generated polynucleotide into a mammal and determining whether an immune response is induced against the cytokine.

40. The method of claim 29, wherein the co-stimulator is B7-1 (CD80) or B7-2 (CD86) and the cell is tested for ability to costimulate an immune response.

41. The method of any of claims 1, 3, or 5, wherein the optimized recombinant polynucleotide encodes a cytokine antagonist.

42. The method of claim 41, wherein the cytokine antagonist is selected from the group consisting of a soluble cytokine receptor and a transmembrane cytokine receptor having a defective signal sequence.

43. The method of claim 41, wherein the cytokine antagonist is selected from the group consisting of Δ IL-1 OR and Δ IL-4R.

44. The method of any of claims 1, 3, or 5, wherein the optimized non-stochastically generated polynucleotide encodes a polypeptide capable of inducing a predominantly T_H1 immune response.

45. The method of any of claims 1, 3, or 5 wherein the optimized non-stochastically generated polynucleotide encodes a polypeptide capable of inducing a predominantly T_H2 immune response.

46. The method of any of claims 1, 3, or 5, wherein said optimized modulatory effect on an immune response is a decrease in an unwanted modulatory effect on an immune response; whereby application of the method can be used to generate a molecule having a decreased ability to elicit an immune response from a host recipient of said molecule, where said recipient can be a human or an animal host; and whereby application of the method can thus be used to generate a molecule having decreased antigenicity with respect to at least one host recipient of said molecule.

47. The method of any of claims 1, 3, or 5, wherein said optimized modulatory effect on an immune response is an increase in a desirable modulatory effect on an immune response; whereby application of the method can be used to generate a molecule having an increased ability to elicit an immune response from a host recipient of said molecule, where said recipient can be a human or an animal host; and whereby application of the method can thus be used to generate a molecule having increased antigenicity with respect to at least one host recipient of said molecule.

48. The method of any of claims 1, 3, or 5, wherein said optimized modulatory effect on an immune response is both a decrease in a first unwanted modulatory effect on an immune response as well as an increase in a second desirable modulatory effect on an immune response; whereby application of the method can be used to generate a molecule having both a decreased ability to elicit a first immune response from a first host recipient of said molecule as well as an increased ability to elicit a second immune response from a second host recipient of said molecule; whereby the first and the second recipient hosts can be the same or different; whereby each of the first and the second recipient hosts can be a human or an animal host; and whereby application of the method can thus be used to generate a molecule having both a first decreased antigenicity with respect to at least one host recipient of said molecule and a second decreased antigenicity with respect to at least one host recipient of said molecule.

49. The method of claim 48, wherein said first and said second modulatory effect on an immune response are evolved for respectively a first and a second module on the same multimodule vaccine vector; whereby a module is exemplified by the following modules, as well as by a fragment derivative or analog thereof: an antigen coding sequence, a polyadenylation sequence, a sequence coding for a co-stimulatory molecule, a sequence coding for an inducible repressor or transactivator, a eukaryotic origin or replication, a prokaryotic origin of replication, a sequence coding for a prokaryotic marker, and enhancer, a promoter, and operator, and an intron.

50. The method of any of claims 1, 3, or 5, wherein the optimized modulatory effect on an immune response is comprised of an increase in the stability of the immunomodulatory (IM) polynucleotide or polypeptide encoded thereby; whereby application of the method can be used to generate a molecule having an increased stability ex vivo, thus, for example, increasing shelf-life and/or ease of storage and/or length of time before expiration of activity upon storage; and whereby application of the method can also be used to generate a molecule having an increased stability in vivo upon administration to a host recipient, thus, for example, increasing resistance to digestive acids and/or increasing stability in the circulation and/or any other method of elimination or destruction by the host recipient.

51. The method of any of claims 1, 3, or 5, wherein the immunomodulatory (IM) polynucleotide or polypeptide encoded thereby; has an optimized modulatory effect on an immune response in a human host recipient as compared with prior to optimization; whereby application of the method can thus be used to generate an optimized genetic vaccine for human recipients.

(IM) polynucleotide or polypeptide encoded thereby; has an optimized modulatory effect on an immune response in an animal host recipient as compared with prior to optimization; whereby application of the method can thus be used to generate an optimized genetic vaccine for animal recipients, including animals that are farmed or raised by man, animals that are not farmed or raised by man, domesticated animals, and non-domesticated animals.

53. A method for obtaining an optimized polynucleotide that encodes an accessory molecule that improves the transport or presentation of antigens by a cell, the method comprising: a) creating a library of non-stochastically generated polynucleotides by subjecting to optimization by non-stochastic directed evolution a parental polynucleotide set in which is encoded all or part of the accessory molecule; and b) screening the library to identify an optimized non-stochastically generated progeny polynucleotide that encodes a recombinant molecule that confers upon a cell an increased or decreased ability to transport or present an antigen on a surface of the cell compared to an accessory molecule encoded by template polynucleotides not subjected to the non-stochastic reassembly; whereby application of the method can thus be used to generate an optimized molecule for human recipients &/or animal recipients, including animals that are farmed or raised by man, animals that are not farmed or raised by man, domesticated animals, and non-domesticated animals; whereby optimization can thus be achieved using one or more of the directed evolution methods as described herein in any combination, permutation, and iterative manner; whereby these directed evolution methods include the introduction of point mutations by non-stochastic methods, including by "gene site saturation mutagenesis" as described herein; and whereby these directed evolution methods also include the introduction mutations by non-stochastic polynucleotide reassembly methods as described herein; including by synthetic ligation polynucleotide reassembly as described herein.

54. The method of claim 53, wherein the screening involves: i) introducing the library of non-stochastically generated polynucleotides into a genetic vaccine vector that encodes an antigen to form a library of vectors; introducing the library of vectors into mammalian cells; and ii) identifying mammalian cells that exhibit increased or decreased immunogenicity to the antigen.

55. The method of claim 53, wherein the accessory molecule comprises a proteasome or a TAP polypeptide.

56. The method of claim 53, wherein the accessory molecule comprises a cytotoxic T-cell inducing sequence.

57. The method of claim 56, wherein the cytotoxic T-cell inducing sequence is obtained from a hepatitis B surface antigen.

58. The method of claim 53, wherein the accessory molecule comprises an immunogenic agonist sequence.

59. A method for obtaining an immunomodulatory polynucleotide that has, an optimized expression in a recombinant expression host, the method comprising: creating a library of non-stochastically generated progeny polynucleotides from a parental polynucleotide set; whereby optimization can thus be achieved using one or more of the directed evolution methods as described herein in any combination, permutation and iterative manner; whereby these directed evolution methods include the introduction of mutations by non-stochastic methods, including by "gene site saturation mutagenesis" as described herein; and whereby these directed evolution methods also include the introduction mutations by non-stochastic polynucleotide reassembly methods as described herein; including by synthetic ligation polynucleotide reassembly as described herein.

60. A method for obtaining an immunomodulatory polynucleotide that has an optimized expression in a recombinant expression host, the method comprising: screening a library of non-stochastically generated progeny polynucleotides to identify an optimized non-stochastically generated progeny polynucleotide that has an optimized expression in a recombinant expression host when compared to the expression of a parental polynucleotide from which the library was created.

61. A method for obtaining an immunomodulatory polynucleotide that has an optimized expression in a recombinant expression host, the method comprising: a) creating a library of non-stochastically generated progeny polynucleotides from a parental polynucleotide set; and b) screening a library of non-stochastically generated progeny polynucleotides to identify an optimized non-stochastically generated progeny polynucleotide that has an optimized expression in a recombinant

polynucleotide from which the library was created; whereby optimization can thus be achieved using one or more of the directed evolution methods as described herein in any combination, permutation, and iterative manner; whereby these directed evolution methods include the introduction of point mutations by non-stochastic methods, including by "gene site saturation mutagenesis" as described herein; and whereby these directed evolution methods also include the introduction mutations by non-stochastic polynucleotide reassembly methods as described herein; including by synthetic ligation polynucleotide reassembly as described herein.

62. The method of any of claims 59-61, wherein the recombinant expression host is a prokaryote.

63. The method of any of claims 59-61, wherein the recombinant expression host is a eukaryote.

64. The method of claim 63, wherein the recombinant expression host is a plant.

65. The method of claim 64, wherein the recombinant expression host is a monocot.

66. The method of claim 64, wherein the recombinant expression host is a dicot.

67. The method of any of claims 1, 3, 5, 53, or 59-61, wherein creating a library of non-stochastically generated progeny polynucleotides from a parental polynucleotide set is comprised of subjecting the parental polynucleotide set to "gene site saturation mutagenesis" as described herein.

68. The method of any of claims 1, 3, 5, 53, or 59-61, wherein creating a library of non-stochastically generated progeny polynucleotides from a parental polynucleotide set is comprised of subjecting the parental polynucleotide set to "synthetic ligation polynucleotide reassembly" as described herein.

69. The method of any of claims 1, 3, 5, 53, or 59-61, wherein creating a library of non-stochastically generated progeny polynucleotides from a parental polynucleotide set is comprised of subjecting the parental polynucleotide set to both "gene site saturation mutagenesis" as described herein, and to "synthetic ligation polynucleotide reassembly" as described herein.

70. The method of claim 1, wherein the directed evolution method is synthetic ligation reassembly.

71. The method of claim 1, wherein the directed evolution method is gene site saturated mutagenesis.

72. The method of claim 1, wherein the directed evolution method is non-stochastic ligation reassembly.

73. The method of claim 1, wherein the directed evolution method is exonuclease-mediated reassembly.

74. The method of claim 1, wherein the directed evolution method is end selection.

75. The method of claim 1, wherein the directed evolution method is shuffling.

76. The method of claim 1, wherein the immunomodulatory polynucleotide encodes a cancer antigen.

77. The method of claim 1, wherein the immunomodulatory polynucleotide encodes a bacterial antigen.

78. The method of claim 1, wherein the immunomodulatory polynucleotide encodes a viral antigen.

79. The method of claim 1, wherein the immunomodulatory polynucleotide encodes a parasite antigen.

80. The method of claim 1, wherein the immunomodulatory polynucleotide encodes a self-antigen.

81. The method of claim 1, wherein the immune response is a humoral immune response.

82. The method of claim 1, wherein the immune response is a cellular

83. The method of claim 1, wherein the immunomodulatory polynucleotide encodes a cytokine.

84. The method of claim 83, wherein the cytokine is IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, GM-CSF, G-CSF, TNF- α , IFN- α , IFN- γ , or IL-20 (MDA-7).

85. The method as in any of claims 1, 3, or 5, wherein the immune response prior to optimization or following optimization is determined in vitro.

86. The method as in any of claims 1, 3, or 5, wherein the immune response prior to optimization or following optimization is determined in vivo.

L5 ANSWER 72 OF 112 USPATFULL on STN

2002:280112 Detection of nucleic acids by target-catalyzed product formation.

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US 2002155548 A1 20021024

APPLICATION: US 2001-989757 A1 20011120 (9)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method is disclosed for modifying an oligonucleotide, which method has application to the detection of a polynucleotide analyte. An oligonucleotide is reversibly hybridized with a polynucleotide, for example, a polynucleotide analyte, in the presence of a 5'-nuclease under isothermal conditions. The polynucleotide analyte serves as a recognition element to enable a 5'-nuclease to cleave the oligonucleotide to provide (i) a first fragment—that is substantially non-hybridizable to the polynucleotide analyte and (ii) a second fragment that lies 3' of the first fragment (in the intact oligonucleotide) and is substantially hybridizable to the polynucleotide analyte. At least a 100-fold molar excess of the first fragment and/or the second fragment are obtained relative to the molar amount of the polynucleotide analyte. The presence of the first fragment and/or the second fragment is detected, the presence thereof indicating the presence of the polynucleotide analyte. The method has particular application to the detection of a polynucleotide analyte such as DNA. Kits for conducting methods in accordance with the present invention are also disclosed.

CLM What is claimed is:

1. A method for modifying an **oligonucleotide**, said method comprising incubating said **oligonucleotide** with a polynucleotide and a 5'-nuclease wherein at least a portion of said **oligonucleotide** is reversibly hybridized to said polynucleotide under isothermal conditions and wherein said **oligonucleotide** is cleaved to provide (i) a first fragment that is substantially non-hybridizable to said polynucleotide and includes no more than one nucleotide from the 5'-end of said portion and (ii) a second fragment that is 3' of said first fragment with reference to the intact **oligonucleotide** and is substantially hybridizable to said polynucleotide.

2. The method of claim 1 wherein the amounts of fragments that are formed are at least 100-fold larger than the amount of said polynucleotide.

3. The method of claim 1 wherein a second **oligonucleotide** is present during said incubating, said second **oligonucleotide** having the characteristic of hybridizing to a site on said polynucleotide that is 3' of the site at which said **oligonucleotide** is reversibly hybridized and of being substantially non-reversibly hybridized to said polynucleotide under said isothermal conditions.

4. The method of claim 3 wherein said second **oligonucleotide** hybridizes to said polynucleotide at a site contiguous with the site on said polynucleotide at which said first **oligonucleotide** reversibly hybridizes.

5. The method of claim 4 wherein the amounts of fragments that are formed are at least 100-fold larger than the amount of said polynucleotide.

6. The method of claim 1 wherein a single nucleoside triphosphate is present during said incubating.

7. A method for detecting a polynucleotide analyte, which comprises: (a) reversibly hybridizing an **oligonucleotide** with a polynucleotide

polynucleotide analyte serves as a recognition element to enable said 5'-nuclease to cleave said **oligonucleotide** to provide (i) a first fragment that is substantially non-hybridizable to said polynucleotide analyte and (ii) a second fragment that lies 3' of said first fragment in the intact **oligonucleotide** and is substantially hybridizable to said polynucleotide analyte wherein at least a 100-fold molar excess of said first fragment and/or said second fragment are obtained relative to the molar amount of said polynucleotide analyte, and (b) detecting the presence of said first fragment and/or said second fragment, the presence thereof indicating the presence of said polynucleotide analyte.

8. The method of claim 7 wherein at least one of said first fragment and said second fragment has a label.

9. The method of claim 7 wherein said first fragment includes no more than 1 nucleotide from the 5'-end of that portion of said **oligonucleotide** that hybridizes to said polynucleotide analyte.

10. The method of claim 7 wherein a second **oligonucleotide** is present during said reversible hybridizing, said second **oligonucleotide** having the characteristic of hybridizing to a site on said polynucleotide analyte that is 3' of the site at which said **oligonucleotide** hybridizes wherein said polynucleotide analyte is substantially fully hybridized to said second **oligonucleotide** under said isothermal conditions.

11. The method of claim 8 wherein said **oligonucleotide** hybridization sites are contiguous.

12. The method of claim 7 wherein a single nucleoside triphosphate is present during said reversible hybridizing.

13. A method for detecting a polynucleotide analyte, said method comprising: (a) providing in combination a medium suspected of containing said polynucleotide analyte, a molar excess, relative to the suspected concentration of said polynucleotide analyte, of a first **oligonucleotide** at least a portion of which is capable of reversibly hybridizing with said polynucleotide analyte under isothermal conditions, a 5'-nuclease, and a second **oligonucleotide** having the characteristic of hybridizing to a site on said polynucleotide analyte that is 3' of the site at which said first **oligonucleotide** hybridizes wherein said polynucleotide analyte is substantially fully hybridized to said second **oligonucleotide** under said isothermal conditions, (b) reversibly hybridizing under said isothermal conditions said polynucleotide analyte and said first **oligonucleotide**, wherein said first **oligonucleotide** is cleaved as a function of the presence of said polynucleotide analyte to provide, in at least a 100-fold molar excess of said polynucleotide analyte, (i) a first fragment that is substantially non-hybridizable to said polynucleotide analyte and/or (ii) a second fragment that is 3' of said first fragment in said first **oligonucleotide** and is substantially hybridizable to said polynucleotide analyte, and (c) detecting the presence of said first fragment and/or said second fragment, the presence thereof indicating the presence of said polynucleotide analyte.

14. The method of claim 13 wherein said first fragment and/or said second fragment has a label.

15. The method of claim 14 wherein said label is selected from the group consisting of a member of a specific binding pair, dyes, fluorescent molecules, chemiluminescers, coenzymes, enzyme substrates, radioactive groups and suspendible particles.

16. The method of claim 13 wherein said polynucleotide analyte is DNA.

17. The method of claim 13 wherein said first fragment includes no more than 1 nucleotide from the 5'-end of that portion of said first **oligonucleotide** that is capable of hybridizing to said polynucleotide analyte.

18. The method of claim 13 wherein said second **oligonucleotide** hybridizes to said polynucleotide at a site contiguous with the site on said polynucleotide at which said first **oligonucleotide** hybridizes.

19. The method of claim 13 wherein a single nucleoside triphosphate is present in said combination during said reversible hybridizing.

20. A method for detecting a DNA analyte, said method comprising: (a) providing in combination a medium suspected of containing said DNA analyte, a first **oligonucleotide** at least a portion of which is capable of reversibly hybridizing with said DNA analyte under isothermal conditions, a 5'-nuclease, and a second **oligonucleotide** having the

of the site at which said first **oligonucleotide** hybridizes wherein said DNA analyte is substantially fully hybridized to said second **oligonucleotide** under said isothermal conditions, (b) reversibly hybridizing said polynucleotide analyte and said first **oligonucleotide** under said isothermal conditions, wherein said first **oligonucleotide** is cleaved to (i) a first fragment that is substantially non-hybridizable to said DNA analyte and (ii) a second fragment that is 3' of said first fragment in said first **oligonucleotide** and is substantially hybridizable to said DNA analyte wherein at least a 100-fold molar excess, relative to said DNA analyte, of said first fragment and/or said second fragment is produced and (c) detecting the presence of said first fragment and/or said second fragment, the presence thereof indicating the presence of said DNA analyte.

21. The method of claim 20 wherein said first **oligonucleotide** has a substituent that facilitates separation of said first fragment or said second fragment from said medium.

22. The method of claim 20 wherein first fragment and/or said second fragment has a label.

23. The method of claim 22 wherein said label is selected from the group consisting of a member of a specific binding pair, dyes, fluorescent molecules, chemiluminescers, coenzymes, enzyme substrates, radioactive groups and suspendible particles.

24. The method of claim 20 wherein a single nucleoside triphosphate is present in said combination during said reversible hybridizing.

25. The method of claim 20 wherein said second **oligonucleotide** hybridizes to said polynucleotide at a site contiguous with the site on said polynucleotide at which said first **oligonucleotide** hybridizes.

26. The method of claim 20 wherein said first **oligonucleotide** and/or said second **oligonucleotide** is DNA.

27. A method for detecting a polynucleotide analyte, said method comprising: (a) providing in combination a medium suspected of containing said polynucleotide analyte, a first DNA **oligonucleotide** at least a portion of which is capable of reversibly hybridizing with said polynucleotide analyte under isothermal conditions, a 5'-nuclease, and a second DNA **oligonucleotide** having the characteristic of hybridizing to a site on said polynucleotide analyte that is 3' of, and contiguous with, the site at which said first DNA **oligonucleotide** hybridizes wherein said polynucleotide analyte is substantially fully hybridized to said second DNA **oligonucleotide** under said isothermal conditions, (b) reversibly hybridizing under said isothermal conditions said polynucleotide analyte and said first DNA **oligonucleotide**, wherein said first DNA **oligonucleotide** is cleaved as a function of the presence of said polynucleotide analyte to provide, in at least a 100-fold molar excess of said polynucleotide analyte, (i) a first fragment that is substantially non-hybridizable to said polynucleotide analyte and/or (ii) a second fragment that is 3' of said first fragment in said first DNA **oligonucleotide** and is substantially hybridizable to said polynucleotide analyte, and (c) detecting the presence of said first fragment and/or said second fragment, the presence thereof indicating the presence of said polynucleotide analyte.

28. The method of claim 27 wherein said first fragment and/or said second fragment has a label.

29. The method of claim 28 wherein said label is selected from the group consisting of a member of a specific binding pair, dyes, fluorescent molecules, chemiluminescers, coenzymes, enzyme substrates, radioactive groups and suspendible particles.

30. The method of claim 27 wherein said polynucleotide analyte is DNA.

31. The method of claim 27 wherein a single nucleoside triphosphate is present in said combination during said reversible hybridizing.

32. A kit for detection of a polynucleotide comprising in packaged combination: (a) a first **oligonucleotide** having the characteristic that, when reversibly hybridized under isothermal conditions to at least a portion of said polynucleotide, it is degraded by a 5'-nuclease to provide (i) a first fragment that is substantially non-hybridizable to said polynucleotide and (ii) a second fragment that is 3' of said first fragment in said first **oligonucleotide** and is substantially hybridizable to said polynucleotide (b) a second **oligonucleotide** having the characteristic of hybridizing to a site on said polynucleotide that is separated by no more than one nucleotide from the 3'-end of the site at which said first **oligonucleotide** hybridizes

second **oligonucleotide** under said isothermal conditions, and (c) a 5'-nuclease.

33. The kit of claim 32 which comprises a single nucleoside triphosphate.

34. The kit of claim 32 wherein said first **oligonucleotide** and said second **oligonucleotide** are DNA.

L5 ANSWER 73 OF 112 USPATFULL on STN

2002:280001 Detection of **dengue virus**.

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US 2002155435 A1 20021024

APPLICATION: US 2002-85944 A1 20020228 (10)

PRIORITY: US 2001-272535P 20010301 (60)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to a pair of **dengue virus**-specific primers for use in a reverse transcriptase-polymerase chain reaction to detect **dengue virus**.

CLM What is claimed is:

1. A method for detecting **dengue virus** comprising: obtaining a sample which is suspected of containing **dengue virus** RNA; performing a reverse transcriptase-polymerase chain reaction on the sample with a first **dengue virus**-specific primer and a second **dengue virus**-specific primer to amplify the **dengue virus** RNA, if present, wherein the first **dengue virus**-specific primer is 18 to 28 nucleotides in length and includes at least 18 consecutive nucleotides of SEQ ID NO: 1, and the second **dengue virus**-specific primer is 18 to 28 nucleotides in length and includes at least 18 consecutive nucleosides of SEQ ID NO: 2; and detecting the amplification product as an indication of presence of **dengue virus** in the sample.

2. The method of claim 1, wherein the first **dengue virus**-specific primer is 18 to 23 nucleotides in length.

3. The method of claim 1, wherein the first **dengue virus**-specific primer is the nucleotide sequence of SEQ ID NO: 1.

4. The method of claim 1, wherein the second **dengue virus**-specific primer is 18 to 23 nucleotides in length.

5. The method of claim 1, wherein the second **dengue virus**-specific primer is the nucleotide sequence of SEQ ID NO: 2.

6. The method of claim 2, wherein the second **dengue virus**-specific primer is 18 to 23 nucleotides in length.

7. The method of claim 3, wherein the second **dengue virus**-specific primer is the nucleotide sequence of SEQ ID NO: 2

8. A method for quantitating **dengue virus** comprising: obtaining a sample which is suspected of containing **dengue virus** RNA, and mixing it with a known amount of a competitor nucleic acid; performing a reverse transcriptase-polymerase chain reaction on the sample and the competitor nucleic acid with a first **dengue virus**-specific primer and a second **dengue virus**-specific primer to amplify the **dengue virus** RNA, if present, and the competitor nucleic acid, wherein the first **dengue virus**-specific primer is 18 to 28 nucleotides in length and includes at least 18 nucleotides of SEQ ID NO: 1, and the second **dengue virus**-specific primer is 18 to 28 nucleotides in length and includes at least 18 nucleosides of SEQ ID NO: 2; and comparing the amounts of the amplification product of the **dengue virus** RNA, if present, to the amplification product of the competitor nucleic acid to quantitate the **dengue virus** RNA in the sample.

9. The method of claim 8, wherein the first **dengue virus**-specific primer is 18 to 23 nucleotides in length.

10. The method of claim 8, wherein the first **dengue virus**-specific primer is the nucleotide sequence of SEQ ID NO: 1.

11. The method of claim 8, wherein the second **dengue virus**-specific primer is 18 to 23 nucleotides in length.

12. The method of claim 8, wherein the second **dengue virus**-specific primer is the nucleotide sequence of SEQ ID NO: 2.

13. The method of claim 9, wherein the second **dengue virus**-specific

14. The method of claim 10, wherein the second **dengue virus-specific primer** is the nucleotide sequence of SEQ ID NO: 2.
15. A kit for detecting **dengue virus** comprising: A first **dengue virus-specific primer**, which is 18 to 28 nucleotides in length and includes at least 18 nucleotides of SEQ ID NO: 1; and A second **dengue virus-specific primer**, which is 18 to 28 nucleotides in length and includes at least 18 nucleotides of SEQ ID NO: 2.
16. The kit of claim 15, further comprising a known amount of a competitor nucleic acid with length detectably different from the **dengue virus** RNA.
17. The kit of claim 15, wherein the first **dengue virus-specific primer** is 18 to 23 nucleotides in length.
18. The kit of claim 15, wherein the first **dengue virus-specific primer** is the nucleotide sequence of SEQ ID NO: 1.
19. The kit of claim 15, wherein the second **dengue virus-specific primer** is 18 to 23 nucleotides in length.
20. The kit of claim 15, wherein the second **dengue virus-specific primer** is the nucleotide sequence of SEQ ID NO: 2.
21. The kit of claim 17, wherein the second **dengue virus-specific primer** is 18 to 23 nucleotides in length.
22. The kit of claim 18, wherein the second **dengue virus-specific primer** is the nucleotide sequence of SEQ ID NO: 2.
23. A nucleic acid, which is 18 to 28 nucleotides in length and includes at least 18 consecutive nucleotides of SEQ ID NO: 1.
24. The nucleic acid of claim 23, wherein the nucleic acid is 18 to 23 nucleotides in length and includes at least 18 consecutive nucleotides of SEQ ID NO: 1.
25. The nucleic acid of claim 23, wherein the nucleic acid is the nucleotide sequence of SEQ ID NO: 1.
26. A nucleic acid, which is 18 to 28 nucleotides in length and includes at least 18 consecutive nucleotides of SEQ ID NO: 2.
27. The nucleic acid of claim 26, wherein the nucleic acid is 18 to 23 nucleotides in length and includes at least 18 consecutive nucleotides of SEQ ID NO: 2.
28. The nucleic acid of claim 26, wherein the nucleic acid is the nucleotide sequence of SEQ ID NO: 2.
29. An isolated nucleic acid comprising a fragment of a dengue viral genome or a DNA copy thereof, wherein the fragment includes: a first sequence that is complementary or identical to at least 18 consecutive nucleotides of SEQ ID NO: 1; a second sequence that is complementary or identical to at least 18 consecutive nucleotides of SEQ ID NO: 2; and a non-naturally occurring deletion or insertion, the deletion or insertion occurring in a region of the fragment flanked by the first and the second sequence.
30. The nucleic acid of claim 29, wherein the first sequence is complementary or identical to SEQ ID NO: 1 and the second sequence that is complementary or identical to SEQ ID NO: 2.

L5 ANSWER 74 OF 112 USPTAFULL on STN

2002:275896 Analysis of genetic polymorphisms and gene copy number.

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US 6468744 B1 20021022

WO 9830883 19980716

APPLICATION: US 1999-341399 19991117 (9)

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention provides methods for detecting variations in polymorphic sites and/or variations in gene copy number. The methods are particularly useful for analysis of biotransformation genes, such as cytochromes P450.

CLM What is claimed is:

1. A method of determining copy number of a gene present in an individual, comprising: analyzing a plurality of polymorphic sites in a chromosome containing a gene from an individual to determine the number of different polymorphic forms present at each site; and assigning the copy number of the gene as the highest number of polymorphic forms present at a single site.
2. The method of claim 1, wherein the plurality of polymorphic sites are in a noncoding segment of the gene.
3. The method of claim 1, wherein the plurality of polymorphic sites are silent polymorphisms.
4. The method of claim 3, wherein the at least one polymorphic site is present in an intronic segment of the gene.
5. The method of claim 1, wherein the plurality of polymorphic sites comprises at least 10 sites.
6. The method of claim 1, wherein the plurality of polymorphic sites comprises at least 50 sites.
7. The method of claim 1, further comprising: obtaining a tissue sample from the individual containing the gene and amplifying the gene or a fragment thereof.
8. The method of claim 1, wherein the analyzing comprises: contacting a nucleic acid comprising the gene or a fragment thereof with an array of **oligonucleotides**, the array comprising a plurality of subarrays, each subarray spanning a polymorphic site and complementarity to at least one polymorphic form of the gene at the site; detecting hybridization intensities of the nucleic acid to the **oligonucleotides** in the array, whereby the pattern of hybridization indicates the number of polymorphic forms present at each polymorphic site.
9. The method of claim 8, wherein the subarrays each comprise a plurality of probe groups, each probe group complementarity to a different polymorphic form at the site.
10. The method of claim 9, wherein a probe group comprises (a) a first probe set comprising a plurality of probes spanning a polymorphic site of the gene, each probe comprising a segment of at least six nucleotides exactly complementary to a polymorphic form of the gene at the site, the segment including at least one interrogation position complementary to a corresponding nucleotide in the polymorphic form, (b) a second probe set comprising a corresponding probe for each probe in the first probe set, the corresponding probe in the second probe set being identical to a sequence comprising the corresponding probe from the first probe set or a subsequence of at least six nucleotides thereof that includes the at least one interrogation position, except that the at least one interrogation position is occupied by a different nucleotide in each of the two corresponding probes from the first and second probe sets.
11. The method of claim 9, wherein a probe group comprises (a) a first probe set comprising a plurality of probes spanning a polymorphic site, each probe comprising a segment of at least six nucleotides exactly complementary to a subsequence of a polymorphic form at the site, the segment including at least one interrogation position complementary to a corresponding nucleotide in the polymorphic form, (b) second, third and fourth probe sets, each comprising a corresponding probe for each probe in the first probe set, the probes in the second, third and fourth probe sets being identical to a sequence comprising the corresponding probe from the first probe set or a subsequence of at least six nucleotides thereof that includes the at least one interrogation position, except that the at least one interrogation position is occupied by a different nucleotide in each of the four corresponding probes from the four probe sets.
12. The method of claim 1, wherein a single polymorphic form is present at each of the plurality of sites and the copy number of the gene is assigned as 1.
13. The method of claim 1, wherein two polymorphic forms are present at one site and a single polymorphic form is present at each other of the plurality of sites, and the copy number of the gene is assigned as 2.

14. The method of claim 1, wherein three polymorphic forms are present at a first polymorphic site, a single polymorphic form is present at a second polymorphic site and two polymorphic forms are present at a third polymorphic site and the copy number of the gene is assigned as 3.

15. The method of claim 1, further comprising analyzing a phenotype-determining polymorphic site in the gene to determine which polymorphic form(s) are present at the site.

16. The method of claim 15, further comprising diagnosing a phenotype of the patient based on the polymorphic form(s) present at the phenotype-determining polymorphic site.

L5 ANSWER 75 OF 112 USPTAFULL on STN

2002:237182 Transgenic animals and cell lines for screening drugs effective for the treatment or prevention of alzheimer's disease.

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US 2002129391 A1 20020912

APPLICATION: US 2001-964412 A1 20010928 (9)

PRIORITY: US 1997-38908P 19970226 (60)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Disclosed are transgenic animals and transfected cell lines expressing a protein associated with Alzheimer's Disease, neuroectodermal tumors, malignant astrocytomas, and glioblastomas. Also disclosed is the use of such transgenic animals and transfected cell lines to screen potential drug candidates for treating or preventing Alzheimer's disease, neuroectodermal tumors, malignant astrocytomas, and glioblastomas. The invention also relates to new antisense oligonucleotides, ribozymes, triplex forming DNA and external guide sequences that can be used to treat or prevent Alzheimer's disease, neuroectodermal tumors, malignant astrocytomas, and glioblastomas.

CLM What is claimed is:

1. A DNA construct, which comprises a DNA molecule of Seq. ID No. 1 or a DNA molecule which is at least 40% homologous thereto, or a fragment thereof, wherein said DNA molecule is under control of a heterologous neuro-specific promoter.

2. The DNA construct of claim 1, which is contained within a vector.

3. The DNA construct of claim 1, which is contained by a viron.

4. The DNA construct of claim 1, wherein said DNA molecule has Seq. ID No. 1.

5. A host cell transformed with the DNA construct of claim 1.

6. The host cell line of claim 5, which is a neuronal cell.

7. A transgenic non-human animal, all of whose germ and somatic cells comprises the DNA molecule of Seq. ID No. 1 or a DNA molecule which is at least 40% homologous thereto.

8. The transgenic non-human animal of claim 7, wherein the DNA molecule contained in each germ and somatic cell has Seq. ID No. 1.

9. The transgenic non-human animal of claim 7, wherein the protein coded for by said DNA molecule is overexpressed in the brain of the animal.

10. An in vitro method for screening a candidate drug that is potentially useful for the treatment or prevention of Alzheimer's disease, neuroectodermal tumors, malignant astrocytomas, and glioblastomas, which comprises (a) contacting a candidate drug with the host cell line of claim 5, and (b) detecting at least one of the following: (i) the suppression or prevention of expression of the protein coded for by the DNA construct; (ii) the increased degradation of the protein coded for by the DNA construct; or (iii) the reduction of frequency of at least one of neuritic sprouting, nerve cell death, degenerating neurons, neurofibrillary tangles, or irregular swollen neurites and axons in the host; due to the drug candidate compared to a control cell line which has not contacted the candidate drug.

11. The method of claim 10, wherein said protein has Seq. ID No. 2.

12. The method of claim 10, wherein said protein is over-expressed by said host cell.

13. The method of claim 10, wherein said cell is a neuronal cell.

14. An in vivo method for screening a candidate drug that is potentially

neuroectodermal tumors, malignant astrocytomas, and glioblastomas, which comprises (a) administering a candidate drug to the transgenic animal of claim 7, and (b) detecting at least one of the following: (i) the suppression or prevention of expression of the protein coded for by the DNA construct contained by said animal; (ii) the increased degradation of the protein coded for by the DNA construct contained by said animal; or (iii) the reduction of frequency of at least one of neuritic sprouting, nerve cell death, degenerating neurons, neurofibrillary tangles, or irregular swollen neurites and axons in the host; due to the drug candidate compared to a control animal which has not received the candidate drug.

15. The method of claim 14, wherein the DNA construct contained by said animal has Seq. ID No. 1.

16. The method of claim 14, wherein the protein coded for by the DNA construct contained by said animal is over-expressed in the brain of said animal.

17. An antisense **oligonucleotide** which is complementary to an NTP mRNA sequence corresponding to nucleotides 150-1139 of Seq. ID No. 1.

18. The antisense **oligonucleotide** of claim 17, which is a 15 to 40-mer.

19. The antisense **oligonucleotide** of claim 17, wherein said antisense **oligonucleotide** is selected from the group consisting of Seq ID Nos. 9 to 11.

20. The antisense **oligonucleotide** of claim 17, which is a deoxyribonucleic acid.

21. The antisense **oligonucleotide** of claim 17, which is a deoxyribonucleic acid phosphorothioate.

22. The antisense **oligonucleotide** of claim 17, which is a derivative of a deoxyribonucleic acid or a deoxyribonucleic acid phosphorothioate.

23. A pharmaceutical composition comprising the antisense **oligonucleotide** of claim 17 and a pharmaceutically acceptable carrier.

24. A ribozyme comprising a target sequence which is complementary to an NTP mRNA sequence corresponding to nucleotides 150-1139 of Seq. ID No. 1.

25. A pharmaceutical composition comprising the ribozyme of claim 24 and a pharmaceutically acceptable carrier.

26. An oligodeoxynucleotide that forms triple stranded regions with the a region of AD7c-NTP coding nucleic acid and having the sequence 3'X5'-L-5'X3', wherein X comprises an AD7c-NTP nucleic acid sequence corresponding to nucleotides 150-1139 of Seq. ID No. 1, and wherein L represents an **oligonucleotide** linker or a bond.

27. A pharmaceutical composition comprising the oligodeoxynucleotide of claim 26 and a pharmaceutically acceptable carrier.

28. An oligodeoxynucleotide that forms triple stranded regions with the a region of AD7c-NTP coding nucleic acid and having the sequence 5'X3'-L-3'X5', wherein X comprises an AD7c-NTP nucleic acid sequence corresponding to nucleotides 150-1139 of Seq. ID No. 1, and wherein L represents an **oligonucleotide** linker or a bond.

29. A pharmaceutical composition comprising the oligodeoxynucleotide of claim 28 and a pharmaceutically acceptable carrier.

30. A ribonucleotide external guide nucleic acid molecule, comprising, a 10-mer nucleotide sequence corresponding to nucleotides 150-1139 of Seq. ID No. 1 fused to a 3'NCCA nucleotide sequence, wherein N is a purine.

31. The ribonucleotide external guide nucleic acid molecule of claim 30 which is selected from the group consisting of any one of Seq. ID Nos. 12 to 14.

32. A pharmaceutical composition comprising the ribonucleotide of claim 30 and a pharmaceutically acceptable carrier.

33. A method for to treat or prevent dementias of the Alzheimer's type of neuronal degeneration; or to treat or prevent neuroectodermal tumors, malignant astrocytomas, or glioblastomas, comprising administering to an animal in need thereof an antisense **oligonucleotide**, a ribozyme, a triple helix-forming **oligonucleotide** or a ribonucleotide external guide sequence of any one of claims 17, 24, 26, 28, or 30.

34. The method of claim 32, wherein said antisense **oligonucleotide**, ribozyme, triple helix-forming **oligonucleotide** or ribonucleotide external guide sequence is administered to said animal as part of a pharmaceutically acceptable carrier.

=> d 15,cbib,ab,clm,76-100

L5 ANSWER 76 OF 112 USPATFULL on STN

2002:199248 THREE DIMENSIONAL STRUCTURES AND MODELS OF FC RECEPTORS AND USES THEREOF.

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US 2002107359 A1 20020808

APPLICATION: US 1999-245764 A1 19990205 (9)

PRIORITY: US 1998-73972P 19980206 (60)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

- AB Disclosed are crystals, crystal structure FcγRIIa protein, three dimensional coordinates of FcγRIIa protein, and structures and models derived from the FcγRIIa structure. Also disclosed are crystals of FcεRI protein and three dimensional coordinates of FcεRI protein monomers and dimers derived from the FcγRIIa structure. Also disclosed are three dimensional coordinates of FcγRIIb proteins and models of FcγRIIb derived from the FcγRIIa structure. The present invention also includes methods to produce such crystals, crystal structures and models. Uses of such crystals, crystal structures and models are also disclosed, including structure based drug design and therapeutic compositions.
- CLM What is claimed is:
1. A model of an Fc receptor (FcR) protein, wherein said model represents a three dimensional structure that substantially conforms to the atomic coordinates of Table 1.
 2. The model of claim 1, wherein said structure substantially conforms to the atomic coordinates and B-values represented by Table 1.
 3. The model of claim 1, wherein said structure is monomeric.
 4. The model of claim 1, wherein said structure is dimeric.
 5. The model of claim 1, wherein said structure substantially conforms to the atomic coordinates of a table selected from the group consisting of Table 2, Table 3, Table 4 and Table 5.
 6. The model of claim 1, wherein at least about 50% of said structure has an average root-mean-square deviation (RMSD) of less than about 1.5 Å for backbone atoms in secondary structure elements in each domain of said structure.
 7. The model of claim 1, wherein at least about 50% of common amino acid side chains between said structure and a structure comprising said atomic coordinates have an average root-mean-square deviation (RMSD) of less than about 1.5 Å.
 8. The model of claim 1, wherein said FcR protein comprises an amino acid sequence that is at least about 25% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:10, SEQ ID NO:11 and SEQ ID NO:12.
 9. The model of claim 1, wherein said FcR protein comprises an amino acid sequence that is at least about 40% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:10, SEQ ID NO:11 and SEQ ID NO:12.
 10. The model of claim 1, wherein said FcR protein comprises an amino acid sequence that is at least about 60% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:10, SEQ ID NO:11 and SEQ ID NO:12.
 11. The model of claim 1, wherein said FcR protein comprises an amino acid sequence selected from the group consisting of: SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, a mutant of any of said amino acid sequences, and an allelic variant of any of said amino acid sequences.

12. The model of claim 1, wherein said FcR protein comprises an amino acid sequence selected from the group consisting of: an amino acid sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13; a mutant of SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 or SEQ ID NO:13; and an allelic variant of SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 or SEQ ID NO:13.

13. The model of claim 1, wherein said FcR protein is selected from the group consisting of FcγRI protein, FcγRIIa protein, FcγRIIb protein, FcγRIIc protein, FcγRIII protein, FcεRI protein, FcαRI protein and structural homologues of any of said FcR proteins.

14. The model of claim 1, wherein said FcR protein is selected from the group consisting of FcγRI protein, FcγRIIa protein, FcγRIIb protein, FcγRIIc protein, FcγRIII protein, FcεRI protein and FcαRI protein.

15. The model of claim 1, wherein said FcR protein is selected from the group consisting of an FcγRIIa protein monomer, an FcγRIIa protein dimer and structural homologues of said FcγRIIa proteins.

16. The model of claim 1, wherein said FcR protein is selected from the group consisting of an FcεRI protein dimer, an FcεRI protein monomer and structural homologues of said FcεRI proteins.

17. The model of claim 1, wherein said FcR protein is selected from the group consisting of an FcγRI protein dimer, an FcγRI protein monomer and structural homologues of said FcγRI protein.

18. The model of claim 1, wherein said FcR protein is selected from the group consisting of an FcγRIIb protein dimer, an FcγRIIb protein monomer and structural homologues of said FcγRIIb protein.

19. The model of claim 1, wherein said FcR protein is selected from the group consisting of an FcγRIIc protein dimer, an FcγRIIc protein monomer and structural homologues of said FcγRIIc protein.

20. The model of claim 1, wherein said FcR protein is selected from the group consisting of an FcγRIIIb protein dimer, an FcγRIIIb protein monomer and structural homologues of said FcγRIIIb protein.

21. The model of claim 1, wherein said FcR protein is selected from the group consisting of an FcαRI protein dimer, an FcαRI protein monomer and structural homologues of said FcαRI protein.

22. The model of claim 1, wherein said atomic coordinates are generated by the method comprising: (a) providing an FcγRIIa protein in crystalline form; (b) generating an electron-density map of said crystalline FcγRIIa protein; and (c) analyzing said electron-density map to produce said atomic coordinates.

23. The model of claim 22, wherein said crystalline FcγRIIa protein is produced by a method comprising: combining FcγRIIa protein with a mother liquor buffer selected from the group consisting of an acetate salt buffer and a sulphate buffer, and inducing crystal formation to produce said crystalline FcγRIIa protein.

24. The model of claim 23, wherein said acetate buffer comprises about 200 mM ammonium acetate, about 100 mM sodium citrate and about 30% PEG 4000, said buffer having a pH of about 5.6.

25. The model of claim 23, wherein said sulphate buffer comprises about 0.1 M HEPES and about 1.5 M lithium sulphate, said buffer having a pH of about 7.5.

26. The model of claim 22, wherein said step of generating an electron-density map comprises analyzing said crystalline FcγRIIa protein by X-ray diffraction.

27. The model of claim 22, wherein said crystalline FcγRIIa protein is derivatized in Di-γ-iodo bis(ethylenediamine) di Platinum(II) nitrate prior to said X-ray diffraction.

28. The model of claim 22, wherein said crystalline FcγRIIa protein is derivatized in about 5 mM Di-γ-iodo bis[ethylenediamine] di Platinum(II) nitrate prior to said X-ray diffraction.
29. The model of claim 1, wherein said model is a computer image generated by a computer-readable medium encoded with a set of three dimensional coordinates of said three dimensional structure, wherein, using a graphical display software program, said three dimensional coordinates create an electronic file that can be visualized on a computer capable of representing said electronic file as a three dimensional image.
30. A computer-assisted method of structure based drug design of bioactive compounds, comprising: a. providing a model of an Fc receptor (FcR) protein, wherein said model represents a three dimensional structure that substantially conforms to the atomic coordinates of Table 1; b. designing a chemical compound using said model; and, c. chemically synthesizing said chemical compound.
31. The method of claim 30, wherein said method further comprises: d. evaluating the bioactivity of said synthesized chemical compound.
32. The method of claim 30, wherein said three dimensional structure comprises the atomic coordinates listed in Table 1.
33. The method of claim 30, wherein said three dimensional structure is dimeric.
34. The method of claim 30, wherein said three dimensional structure comprises the atomic coordinates listed in a table selected from the group consisting of Table 2, Table 3, Table 4, and Table 5.
35. The method of claim 30, wherein said model comprises a computer image generated when the atomic coordinates listed in Table 1 are analyzed on a computer using a graphical display software program to create an electronic file of said image and visualizing said electronic file on a computer capable of representing said electronic file as a three dimensional image.
36. The method of claim 30, wherein said step of designing comprises computational screening of one or more databases of chemical compounds in which the three dimensional structure of said compounds are known.
37. The method of claim 36, further comprising interacting a compound identified by said screening step with said model by computer.
38. The method of claim 30, wherein said step of designing comprises directed drug design.
39. The method of claim 30, wherein said step of designing comprises random drug design.
40. The method of claim 30, wherein said step of designing comprises grid-based drug design.
41. The method of claim 30, wherein said step of designing comprises selecting compounds which are predicted to mimic said three dimensional structure of said FcR protein.
42. The method of claim 30, wherein said step of designing comprises selecting compounds which are predicted to bind to said three dimensional structure of said FcR protein.
43. The method of claim 30, wherein said bioactivity is selected from the group consisting of inhibiting binding of said FcR protein to an immunoglobulin protein, binding to said FcR protein, binding to an immunoglobulin which is capable of binding to said FcR protein, inhibiting phagocytosis of said immunoglobulin protein, inhibiting dimerization of said FcR protein, stimulating cellular signal transduction through said FcR protein, and stimulating release of cytokines through said FcR protein.
44. The method of claim 30, wherein said FcR protein is FcγRIIa and said bioactivity is selected from the group consisting of inhibiting binding of FcγRIIa protein to IgG, inhibiting phagocytosis of IgG, inhibiting dimerization of FcγRIIa protein, stimulating cellular signal transduction through an FcγRIIa protein, stimulating release of cytokines selected from the group consisting of IL-6 and IL-12.
45. The method of claim 30, wherein said FcR protein is FcγRIIb

binding of FcγRIIIb protein to IgG, inhibiting phagocytosis of IgG, inhibiting dimerization of FcγRIIIb protein, stimulating cellular signal transduction through an FcγRIIIb protein, stimulating release of cytokines selected from the group consisting of IL-6 and IL-12.

46. The method of claim 30, wherein said FcR protein is FcεRI and said bioactivity is selected from the group consisting of inhibiting binding of FcεRI protein to IgE, inhibiting phagocytosis of IgE, inhibiting dimerization of FcεRI protein, stimulating cellular signal transduction through an FcεRI protein, stimulating release of histamine and serotonin by mast cells and inhibiting release of histamine and serotonin by mast cells.

47. A computer-assisted method of structure based drug design of bioactive compounds, comprising: a. providing a model of an Fc receptor (FcR) protein, wherein said model represents a three dimensional structure that substantially conforms to the atomic coordinates selected from the group consisting of atomic coordinates represented by Table 1; atomic coordinates represented by Table 2; atomic coordinates represented by Table 3; atomic coordinates represented by Table 4; and atomic coordinates represented by Table 5; b. designing a chemical compound using said model; and, c. chemically synthesizing said chemical compound.

48. A computer-assisted method of structure based drug design of bioactive compounds, comprising: a. providing a model of a three dimensional structure of an Fc receptor (FcR) protein selected from the group consisting of FcγRIIa, FcγRIIIb and FcεRI; b. designing a chemical compound using said model; and, c. chemically synthesizing said chemical compound.

49. A three dimensional computer image of the three dimensional structure of an FcR protein.

50. The image of claim 49, wherein said structure substantially conforms with the three dimensional coordinates selected from the group consisting of the three dimensional coordinates listed in Table 1; the three dimensional coordinates listed in Table 2; the three dimensional coordinates listed in Table 3; the three dimensional coordinates listed in Table 4; and the three dimensional coordinates listed in Table 5.

51. The image of claim 49, wherein said computer image is generated when a set of three dimensional coordinates comprising said three dimensional coordinates are analyzed on a computer using a graphical display software program to create an electronic file of said image and visualizing said electronic file on a computer capable of representing electronic file as a three dimensional image.

52. The image of claim 49, wherein said three dimensional computer image is represented by a two dimensional image selected from the group consisting of FIG. 4, FIG. 6, FIG. 7, FIG. 8, FIG. 9, FIG. 10, FIG. 14, FIG. 15 and FIG. 16.

53. The image of claim 49, wherein said three dimensional computer image is used to design a therapeutic compound.

54. A computer-readable medium encoded with a set of three dimensional coordinates of an FcR protein having a three dimensional structure that substantially conforms to the atomic coordinates of Table 1, wherein, using a graphical display software program, said three dimensional coordinates create an electronic file that can be visualized on a computer capable of representing said electronic file as a three dimensional image.

55. A computer-readable medium encoded with a set of three dimensional coordinates selected from the group consisting of the three dimensional coordinates represented in Table 1, the three dimensional coordinates represented in Table 2, the three dimensional coordinates represented in Table 3, the three dimensional coordinates represented in Table 4, and the three dimensional coordinates represented in Table 5, wherein, using a graphical display software program, said three dimensional coordinates create an electronic file that can be visualized on a computer capable of representing said electronic file as a three dimensional image.

56. A model of the three dimensional structure of an FcR protein selected from the group consisting of FcγRI protein, FcγRIIb protein, FcγRIIc protein, FcγRIIIb protein, FcεRI protein and FcαRI protein, said model being produced by the method comprising: (a) providing an amino acid sequence of an FcγRIIa

structurally conserved regions shared between said FcγRIIa amino acid sequence and said FcR protein amino acid sequence; and (c) determining atomic coordinates for said FcR protein by assigning said structurally conserved regions of said FcR protein to a three dimensional structure using a three dimensional structure of said FcγRIIa protein which substantially conforms to the atomic coordinates represented in Table 1, to derive a model of said three dimensional structure of said FcR protein amino acid sequence.

57. The model of claim 56, wherein said FcγRI protein amino acid sequence comprises SEQ ID NO:7; wherein said FcγRIIb protein amino acid sequence comprises SEQ ID NO:5; wherein said FcγRIIc protein amino acid sequence comprises SEQ ID NO:6; wherein said FcγRIIIb protein amino acid sequence comprises SEQ ID NO:8; wherein said FcεRI protein amino acid sequence comprises SEQ ID NO:9; and wherein said FcαRI protein amino acid sequence comprises SEQ ID NO:13.

58. A therapeutic composition that, when administered to an animal, reduces IgG-mediated tissue damage, said therapeutic composition comprising an inhibitory compound that inhibits the activity of an Fcγ receptor (FcγR) protein, said inhibitory compound being identified by the method comprising: (a) providing a three dimensional structure of an FcγR protein selected from the group consisting of FcγRI, FcγRIIa, FcγRIIb, FcγRIIc and FcγRIIIb, wherein said three dimensional structure of said FcγR protein substantially conforms to atomic coordinates represented by Table 1; (b) using said three dimensional structure of said FcγR protein to design a chemical compound selected from the group consisting of a compound that inhibits binding of FcγR protein to IgG, a compound that substantially mimics the three dimensional structure of FcγR protein and a compound that inhibits binding of FcγR protein with a molecule that stimulates cellular signal transduction through an FcγR protein; (c) chemically synthesizing said chemical compound; and (d) evaluating the ability of said synthesized chemical compound to reduce IgG-mediated tissue damage.

59. The composition of claim 58, wherein said IgG-mediated tissue damage results from a biological response selected from the group consisting of IgG-mediated hypersensitivity, IgG-mediated recruitment of inflammatory cells, and IgG-mediated release of inflammatory modulators.

60. The composition of claim 58, wherein said structure substantially conforms with the atomic coordinates represented in Table 1.

61. The composition of claim 58, wherein said chemical compound is selected from the group consisting of an inorganic compound and an organic compound.

62. The composition of claim 58, wherein said chemical compound is selected from the group consisting of **oligonucleotides**, peptides, peptidomimetic compounds and small organic molecules.

63. The composition of claim 58, wherein said chemical compound is selected from the group consisting of an analog of said FcγR protein, a substrate analog of said FcγR protein and a peptidomimetic compound of said FcγR protein.

64. The composition of claim 58, wherein said composition further comprises a component selected from the group consisting of an excipient, an adjuvant, and a carrier.

65. A therapeutic composition that, when administered to an animal, enhances IgG-mediated responses, said therapeutic composition comprising a stimulatory compound that stimulates the activity of an Fcγ receptor (FcγR) protein, said stimulatory compound being identified by the method comprising: (a) providing a three dimensional structure of an FcγR protein selected from the group consisting of FcγRI, FcγRIIa, FcγRIIb, FcγRIIc and FcγRIIIb, wherein said three dimensional structure of said FcγR protein substantially conforms to atomic coordinates represented by Table 1; (b) using said three dimensional structure of said FcγR protein to design a chemical compound selected from the group consisting of a compound that stimulates binding of FcγR protein to IgG, a compound that substantially mimics the three dimensional structure of FcγR protein and a compound that stimulates binding of FcγR protein with a molecule that stimulates

chemically synthesizing said chemical compound; and (d) evaluating the ability of said synthesized chemical compound to enhance IgG-mediated responses.

66. A therapeutic composition that, when administered to an animal, reduces IgE-mediated responses, said therapeutic composition comprising an inhibitory compound that inhibits the activity of an Fcε receptor I (FcεRI) protein, said inhibitory compound being identified by the method comprising: (a) providing a three dimensional structure of an FcεRI protein, wherein said three dimensional structure of said FcεRI protein substantially conforms to the atomic coordinates selected from the group consisting of the atomic coordinates represented by Table 1, the atomic coordinates represented by Table 2, the atomic coordinates represented by Table 3, the atomic coordinates represented by Table 4 and the atomic coordinates represented by Table 5; (b) using said three dimensional structure of said FcεRI protein to design a chemical compound selected from the group consisting of a compound that inhibits binding of FcεRI protein to IgE, a compound that substantially mimics the three dimensional structure of FcεRI protein and a compound that inhibits binding of FcεRI protein with a molecule that stimulates cellular signal transduction through an FcεRI protein; (c) chemically synthesizing said chemical compound; and (d) evaluating the ability of said synthesized chemical compound to reduce IgE-mediated responses.

67. The composition of claim 66, wherein said IgE-mediated response results from a biological response selected from the group consisting of IgE-mediated hypersensitivity, IgE-mediated recruitment of inflammatory cells, and IgE-mediated release of inflammatory modulators.

68. The composition of claim 66, wherein said structure comprises the atomic coordinates represented in Table 3.

69. The composition of claim 66, wherein said structure comprises the atomic coordinates represented in Table 4.

70. The composition of claim 66, wherein said chemical compound is selected from the group consisting of an inorganic compound and an organic compound.

71. The composition of claim 66, wherein said chemical compound is selected from the group consisting of **oligonucleotides**, peptides, peptidomimetic compounds and small organic molecules.

72. The composition of claim 66, wherein said chemical compound is selected from the group consisting of an analog of said FcεR protein, a substrate analog of said FcεRI protein and a peptidomimetic compound of said FcεRI protein.

73. The composition of claim 66, wherein said composition further comprises a component selected from the group consisting of an excipient, an adjuvant, and a carrier.

74. A therapeutic composition that, when administered to an animal, enhances IgE-mediated responses, said therapeutic composition comprising a stimulatory compound that stimulates the activity of an Fcε receptor I (FcεRI) protein, said stimulatory compound being identified by the method comprising: (a) providing a three dimensional structure of an FcεRI protein, wherein said three dimensional structure of said FcεRI protein substantially conforms to the atomic coordinates selected from the group consisting of the atomic coordinates represented by Table 1, the atomic coordinates represented by Table 2, the atomic coordinates represented by Table 3, the atomic coordinates represented by Table 4 and the atomic coordinates represented by Table 5; (b) using said three dimensional structure of said FcεRI protein to design a chemical compound selected from the group consisting of a compound that stimulates binding of FcεRI protein to IgE, a compound that substantially mimics the three dimensional structure of FcεRI protein and a compound that stimulates binding of FcεRI protein with a molecule that stimulates cellular signal transduction through an FcεRI protein; (c) chemically synthesizing said chemical compound; and (d) evaluating the ability of said synthesized chemical compound to enhance IgE-mediated responses.

75. A method to determine a three dimensional structure of an FcR protein, said method comprising (a) providing an amino acid sequence of

protein, FcγRIIb protein, FcγRIIc protein, FcγRIIIb protein, FcεRI protein and FcαRI protein, wherein the three dimensional structure of said FcR protein is not known; (b) analyzing the pattern of folding of said amino acid sequence in a three dimensional conformation by fold recognition; and (c) comparing said pattern of folding of said FcR protein amino acid sequence with the three dimensional structure of FcγRIIa protein to determine the three dimensional structure of said FcR protein, wherein said three dimensional structure of said FcγRIIa protein substantially conforms to the atomic coordinates represented in Table 1.

76. A method to derive a model of the three dimensional structure of an FcR protein, said method comprising the steps of: (a) providing an amino acid sequence of an FcγRIIa protein and an amino acid sequence of an FcR protein; (b) identifying structurally conserved regions shared between said FcγRIIa amino acid sequence and said FcR protein amino acid sequence; (c) determining atomic coordinates for said target structure by assigning said structurally conserved regions of said FcR protein to a three dimensional structure using a three dimensional structure of an FcγRIIa protein based on atomic coordinates that substantially conform to the atomic coordinates represented in Table 1 to derive a model of the three dimensional structure of said FcR protein amino acid sequence.

77. The method of claim 76, further comprising assigning atomic coordinates for side chains of said FcR protein by determining sterically allowable positions using a library of rotamers.

78. A method to derive a three dimensional structure of a crystallized FcR protein, said method comprising the steps of: (a) comparing the Patterson function of a crystallized FcR protein with the Patterson function of crystalline FcγRIIa protein to produce an electron-density map of said crystallized FcR protein; and (b) analyzing said electron-density map to produce said three dimensional structure of said crystallized FcR protein.

79. The method of claim 78, further comprising the step of electronically simulating said three dimensional structure of said crystallized FcR protein to derive a computer image of said three dimensional structure of said crystallized FcR protein.

80. The method of claim 78, further comprising the step of rotating said Patterson function of said crystallized FcR protein on said Patterson function of said crystalline FcγRIIa protein to determine the correct orientation of said crystallized FcR protein in a crystal of said crystallized FcR protein to identify the initial phases of said crystallized FcR protein.

81. A composition comprising FcγRIIa protein in a crystalline form.

L5 ANSWER 77 OF 112 USPTAFULL on STN

2002:193030 Transgenic animals and cell lines for screening drugs effective for the treatment or prevention of alzheimer's disease.

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US 2002104108 A1 20020801

APPLICATION: US 2001-964666 A1 20010928 (9)

PRIORITY: US 1997-38908P 19970226 (60)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Disclosed are transgenic animals and transfected cell lines expressing a protein associated with Alzheimer's Disease, neuroectodermal tumors, malignant astrocytomas, and glioblastomas. Also disclosed is the use of such transgenic animals and transfected cell lines to screen potential drug candidates for treating or preventing Alzheimer's disease, neuroectodermal tumors, malignant astrocytomas, and glioblastomas. The invention also relates to new antisense oligonucleotides, ribozymes, triplex forming DNA and external guide sequences that can be used to treat or prevent Alzheimer's disease, neuroectodermal tumors, malignant astrocytomas, and glioblastomas.

CLM What is claimed is:

1. A DNA construct, which comprises a DNA molecule of Seq. ID No. 1 or a DNA molecule which is at least 40% homologous thereto, or a fragment thereof, wherein said DNA molecule is under control of a heterologous neuro-specific promoter.

2. The DNA construct of claim 1, which is contained within a vector.

4. The DNA construct of claim 1, wherein said DNA molecule has Seq. ID No. 1.
5. A host cell transformed with the DNA construct of claim 1.
6. The host cell line of claim 5, which is a neuronal cell.
7. A transgenic non-human animal, all of whose germ and somatic cells comprises the DNA molecule of Seq. ID No. 1 or a DNA molecule which is at least 40% homologous thereto.
8. The transgenic non-human animal of claim 7, wherein the DNA molecule contained in each germ and somatic cell has Seq. ID No. 1.
9. The transgenic non-human animal of claim 7, wherein the protein coded for by said DNA molecule is overexpressed in the brain of the animal.
10. An in vitro method for screening a candidate drug that is potentially useful for the treatment or prevention of Alzheimer's disease, neuroectodermal tumors, malignant astrocytomas, and glioblastomas, which comprises (a) contacting a candidate drug with the host cell line of claim 5, and (b) detecting at least one of the following: (i) the suppression or prevention of expression of the protein coded for by the DNA construct; (ii) the increased degradation of the protein coded for by the DNA construct; or (iii) the reduction of frequency of at least one of neuritic sprouting, nerve cell death, degenerating neurons, neurofibrillary tangles, or irregular swollen neurites and axons in the host; due to the drug candidate compared to a control cell line which has not contacted the candidate drug.
11. The method of claim 10, wherein said protein has Seq. ID No. 2.
12. The method of claim 10, wherein said protein is over-expressed by said host cell.
13. The method of claim 10, wherein said cell is a neuronal cell.
14. An in vivo method for screening a candidate drug that is potentially useful for the treatment or prevention of Alzheimer's disease, neuroectodermal tumors, malignant astrocytomas, and glioblastomas, which comprises (a) administering a candidate drug to the transgenic animal of claim 7, and (b) detecting at least one of the following: (i) the suppression or prevention of expression of the protein coded for by the DNA construct contained by said animal; (ii) the increased degradation of the protein coded for by the DNA construct contained by said animal; or (iii) the reduction of frequency of at least one of neuritic sprouting, nerve cell death, degenerating neurons, neurofibrillary tangles, or irregular swollen neurites and axons in the host; due to the drug candidate compared to a control animal which has not received the candidate drug.
15. The method of claim 14, wherein the DNA construct contained by said animal has Seq. ID No. 1.
16. The method of claim 14, wherein the protein coded for by the DNA construct contained by said animal is over-expressed in the brain of said animal.
17. An antisense **oligonucleotide** which is complementary to an NTP mRNA sequence corresponding to nucleotides 150-1139 of Seq. ID No. 1.
18. The antisense **oligonucleotide** of claim 17, which is a 15 to 40-mer.
19. The antisense **oligonucleotide** of claim 17, wherein said anti sense **oligonucleotide** is selected from the group consisting of Seq ID Nos. 9 to 11.
20. The antisense **oligonucleotide** of claim 17, which is deoxyribonucleic acid.
21. The anti sense **oligonucleotide** of claim 17, which is a deoxyribonucleic acid phosphorothioate.
22. The antisense **oligonucleotide** of claim 17, which is a derivative of a deoxyribonucleic acid or a deoxyribonucleic acid phosphorothioate.
23. A pharmaceutical composition comprising the antisense **oligonucleotide** of claim 17 and a pharmaceutically acceptable carrier.
24. A ribozyme comprising a target sequence which is complementary to an NTP mRNA sequence corresponding to nucleotides 150-1139 of Seq. ID No.

25. A pharmaceutical composition comprising the ribozyme of claim 24 and a pharmaceutically acceptable carrier.

26. An oligodeoxynucleotide that forms triple stranded regions with the a region of AD7c-NTP coding nucleic acid and having the sequence 3'X5'-L-5'X3', wherein X comprises an AD7c-NTP nucleic acid sequence corresponding to nucleotides 150-1139 of Seq. ID No. 1, and wherein L represents an **oligonucleotide** linker or a bond.

27. A pharmaceutical composition comprising the oligodeoxynucleotide of claim 26 and a pharmaceutically acceptable carrier.

28. An oligodeoxynucleotide that forms triple stranded regions with the a region of AD7c-NTP coding nucleic acid and having the sequence 5'X3'-L-3'X5', wherein X comprises an AD7c-NTP nucleic acid sequence corresponding to nucleotides 150-1139 of Seq. ID No. 1, and wherein L represents an **oligonucleotide** linker or a bond.

29. A pharmaceutical composition comprising the oligodeoxynucleotide of claim 28 and a pharmaceutically acceptable carrier.

30. A ribonucleotide external guide nucleic acid molecule, comprising, a 10-mer nucleotide sequence corresponding to nucleotides 150-1139 of Seq. ID No. 1 fused to a 3'NCCA nucleotide sequence, wherein N is a purine.

31. The ribonucleotide external guide nucleic acid molecule of claim 30 which is selected from the group consisting of any one of Seq. ID Nos. 12 to 14.

32. A pharmaceutical composition comprising the ribonucleotide of claim 30 and a pharmaceutically acceptable carrier.

33. A method for to treat or prevent dementias of the Alzheimer's type of neuronal degeneration; or to treat or prevent neuroectodermal tumors, malignant astrocytomas, or glioblastomas, comprising administering to an animal in need thereof an antisense **oligonucleotide**, a ribozyme, a triple helix-forming **oligonucleotide** or an ribonucleotide external guide sequence of any one of claims 17, 24, 26, 28, or 30.

34. The method of claim 32, wherein said antisense **oligonucleotide**, ribozyme, triple helix-forming **oligonucleotide** or ribonucleotide external guide sequence is administered to said animal as part of a pharmaceutically acceptable carrier.

L5 ANSWER 78 OF 112 USPTAFULL on STN

2002:116000 Electrochemical detection of nucleic acid sequences.

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US 6391558 B1 20020521

APPLICATION: US 2000-549853 20000414 (9)

PRIORITY: US 1997-40949P 19970318 (60)

DOCUMENT TYPE: Utility; GRANTED.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB An electrochemical detection system which specifically detects selected nucleic acid segments is described. The system utilizes biological probes such as nucleic acid or peptide nucleic acid probes which are complementary to and specifically hybridize with selected nucleic acid segments in order to generate a measurable current when an amperometric potential is applied. The electrochemical signal can be quantified.

CLM What is claimed is:

1. A method of detecting a target molecule having at least one selected nucleic acid or protein segment, comprising: (a) interacting a bioreporter molecule attached to a working electrode surface with the target molecule to form a complex; (b) adding a reporter molecule that can be electrochemically oxidized or reduced upon interaction with said complex; (c) applying a pulse of potential to the working electrode that electrochemically oxidizes or reduces the reporter molecule; (d) repeating step (c); and (e) measuring the current close to the end of step (c) wherein said current is indicative of the presence of the target molecule.

sensors, each sensor comprising a potentiostat, a working electrode with attached target molecule and a reference electrode comprised within a suitable circuit.

3. The method of claim 1 wherein step (e) is repeated and an average or summation signal is determined.

4. The method of claim 1 wherein each sensor of an array of sensors comprises a working electrode and a reference electrode comprised within a suitable circuit.

5. The method of claim 1 further comprising determining a concentration of said target molecule comprising relating a signal from a standard concentration of the target molecule to the signal determined from the target molecule in a sample.

6. The method of claim 1 wherein the intermittent pulse is applied for a period of from 0.1 millisecond to about 100 milliseconds.

7. The method of claim 1 wherein intermittent pulse separation time is from 1 millisecond to about 10 seconds.

8. A method of detecting a target molecule having at least one selected nucleic acid or protein segment, comprising: (a) interacting a bioreporter molecule attached to a working electrode surface with the target molecule to form a complex; (b) adding a reporter molecule that can be electrochemically reduced or oxidized upon interaction with said complex; (c) applying a first potential to the working electrode at or close to the open circuit potential; (d) measuring current close to the end of step (c); (e) applying a second potential or potential pulse to the working electrode that electrochemically oxidizes or reduces the reporter molecule at or near the electrode surface; (f) measuring a current close to the end of step (e); and (g) determining a signal by subtracting the current measured in step (d) from the current measured in step (f); wherein the signal indicates the presence of said target molecule.

9. The method of claim 8 wherein steps (c) through (g) are repeated and a signal is determined from individual signals determined in step (g) by averaging or summation.

10. The method of claim 8 further comprising determining a concentration of said target molecule comprising relating a signal from a standard concentration of the target molecule to the signal determined from the target molecule in a sample.

11. The method of claim 8 wherein the detecting is with an array of sensors, each sensor comprising a potentiostat, the working electrode with attached target molecule and a reference electrode comprised within a suitable circuit.

12. The method of claim 8 wherein the potential pulse separation time is from 1 millisecond to about 10 seconds.

13. The method of claim 1 or claim 8 wherein the nucleic acid target molecule is an **oligonucleotide** selected from the group consisting of DNA, RNA or a peptide nucleic acid.

14. The method of claim 1 or claim 8 wherein the protein target molecule is a protein, peptide or antigen.

15. The method of claim 1 or 8 for detecting a nucleic acid target molecule wherein the bioreporter molecule is complementary to a first selected region of the target nucleic acid segment attached to the surface of the working electrode and wherein the reporter molecule complementary to a second selected region of the target nucleic acid segment is labeled.

16. The method of claim 15 wherein the bioreporter molecule is attached to the working electrode by adsorption, crosslinking, covalent bonding, or charge-charge interaction.

17. The method of claim 16 wherein the bioreporter molecule is attached to the working electrode with avidin, streptavidin, protein G, protein A, neutravidin or antibody.

18. The method of claim 15 wherein the reporter molecule is labeled with fluorescein, digoxigenin, HRP or alkaline phosphatase.

19. The method of claim 15 further comprising: (a) incubating the target molecule and the bioreporter molecule under conditions to form a **primer** extended molecule; and (b) detecting the presence of the

20. The method of claim 19 wherein the **primer** extended molecule is labeled.
21. The method of claim 19 wherein a single **primer** is used.
22. The method of claim 19 wherein the bioreporter is extended before binding to the working electrode.
23. The method of claim 15 wherein the bioreporter has a blocked 3'-end.
24. The method of claim 15 wherein the detector probe comprises a 3'-hydroxyl group.
25. The method of claim 1 or claim 8 wherein the target molecule is a nucleic acid obtained from a DNA sequence comprising a deletion, insertion or single base alteration.
26. The method of claim 19 wherein the **primer** extended molecule is formed by enzyme catalysis.
27. The method of claim 26 wherein the enzyme is a DNA polymerase, an RNA polymerase or a reverse transcriptase.

L5 ANSWER 79 OF 112 USPTAFULL on STN

2002:75204 Detection of nucleic acids by target-catalyzed formation.

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US 6368803 B1 20020409

APPLICATION: US 2000-608721 20000630 (9)

DOCUMENT TYPE: Utility; GRANTED.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method is disclosed for modifying an oligonucleotide, which method has application to the detection of a polynucleotide analyte. An oligonucleotide is reversibly hybridized with a polynucleotide, for example, a polynucleotide analyte, in the presence of a 5'-nuclease under isothermal conditions. The polynucleotide analyte serves as a recognition element to enable a 5'-nuclease to cleave the oligonucleotide to provide (i) a first fragment that is substantially non-hybridizable to the polynucleotide analyte and (ii) a second fragment that lies 3' of the first fragment (in the intact oligonucleotide) and is substantially hybridizable to the polynucleotide analyte. At least a 100-fold molar excess of the first fragment and/or the second fragment are obtained relative to the molar amount of the polynucleotide analyte. The presence of the first fragment and/or the second fragment is detected, the presence thereof indicating the presence of the polynucleotide analyte. The method has particular application to the detection of a polynucleotide analyte such as DNA. Kits for conducting methods in accordance with the present invention are also disclosed.

CLM What is claimed is:

1. A kit for detection of a polynucleotide comprising in packaged combination: (a) a first **oligonucleotide** having the characteristic that, when reversibly hybridized under isothermal conditions to at least a portion of said polynucleotide, it is degraded by a 5'-nuclease to provide (i) a first fragment that is substantially non-hybridizable to said polynucleotide and (ii) a second fragment that is 3' of said first fragment in said first **oligonucleotide** and is substantially hybridizable to said polynucleotide, wherein said isothermal conditions are at or near the melting temperature of a duplex comprising the **oligonucleotide** hybridized to the polynucleotide, (b) a second **oligonucleotide** having the characteristic of hybridizing to a site on said polynucleotide that is separated by no more than one nucleotide from the 3'-end of the site at which said first **oligonucleotide** wherein said polynucleotide is substantially fully hybridized to said second **oligonucleotide** under said isothermal conditions, and (c) a 5'-nuclease.
2. The kit of claim 1 which comprises a single nucleoside triphosphate.
3. The kit of claim 1 wherein said first **oligonucleotide** and said second **oligonucleotide** are DNA.

L5 ANSWER 80 OF 112 USPTAFULL on STN

2002:69768 Quantitative determination of nucleic acid amplification products.

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CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to a method for detecting the amount of a target polynucleotide in a sample. A combination is provided in a medium. The combination comprises (i) a sample suspected of containing the target polynucleotide, the target polynucleotide being in single stranded form, (ii) a reference polynucleotide comprising a sequence that is common with a sequence of the target polynucleotide, and (iii) a predetermined amount of an oligonucleotide probe that has a sequence that hybridizes with the sequence that is common. The combination is subjected to conditions for amplifying the target polynucleotide and the reference polynucleotide. The conditions permit formation of substantially non-dissociative complexes of the target polynucleotide and the reference polynucleotide, respectively, with the oligonucleotide probe. Furthermore, the predetermined amount of the oligonucleotide probe is less than the expected amount of the amplified target polynucleotide. The ratio of the amount of the complex of the target polynucleotide with the oligonucleotide probe to the amount of the complex of the reference polynucleotide with the oligonucleotide probe is determined. Determination of the ratio is facilitated by employing second and third oligonucleotide probes. The second oligonucleotide probe has a sequence that hybridizes only with the second sequence of the target polynucleotide. The third oligonucleotide probe has a sequence that hybridizes only with a respective second sequence of the reference polynucleotide. The ratio is related to the known amount of the reference polynucleotide to determine the amount of the target polynucleotide in the sample. One or more reference polynucleotides may be employed with a corresponding third oligonucleotide probe for each reference probe. Kits for carrying out the above methods are also disclosed. The method is particularly applicable to the amplification and detection of RNA.

CLM What is claimed is:

1. A method for detecting the amount of a target polynucleotide in a sample, said method comprising: (a) providing in single reaction medium (i) a sample suspected of containing said target polynucleotide, said target polynucleotide being in single stranded form, (ii) predetermined amounts of one or more reference polynucleotides, each of said reference polynucleotides comprising a first sequence that is common with a first sequence of said target polynucleotide and a second sequence that is different from a second sequence of said target polynucleotide, (iii) a predetermined amount of a first **oligonucleotide** probe that has a sequence that hybridizes with said sequence that is common, (iv) a second **oligonucleotide** probe that has a sequence that hybridizes only with said second sequence of said target polynucleotide, and (v) one or more third **oligonucleotide** probes, each of said third **oligonucleotide** probes having a sequence that hybridizes only with a respective second sequence of one of said reference polynucleotide, (b) subjecting said single reaction medium to isothermal conditions for amplifying with equal efficiency said target polynucleotide and said one or more reference polynucleotides, wherein said first probe is not extended, and wherein said conditions permit formation of a substantially non-dissociative first termolecular complex of said target polynucleotide, said first **oligonucleotide** probe and said second **oligonucleotide** probe and a substantially non-dissociative second termolecular complex of each of said reference polynucleotide with said first **oligonucleotide** probe and a respective third **oligonucleotide** probe and wherein said predetermined amount of said first **oligonucleotide** probe is less than the expected amount of said amplified target polynucleotide, (c) determining the ratio of the amount of said first termolecular complex to the amount of each of said second termolecular complexes, and (d) relating each of said ratios to the predetermined amount of each of said reference polynucleotides to determine the amount of said target polynucleotide in said sample.

2. The method of claim 1 wherein said first termolecular complex has a first signal producing system and each of said second termolecular complexes has a second signal producing system wherein a signal produced by said first signal producing system is different from a signal produced by each of said second signal producing systems.

3. The method of claim 2 wherein the ratio of said signals is determined and related to the predetermined amount of said reference polynucleotide to determine the amount of said target polynucleotide in said sample.

4. The method of claim 2 wherein said signal producing systems comprise labels selected from the group consisting of a luminescent energy donor and acceptor pair, a singlet oxygen generator and chemiluminescent reactant pair, and an enzyme pair wherein a product of the first enzyme serves as a substrate for the second enzyme.

5. The method of claim 1 wherein said amplification is selected from the

Q β -replicase.

6. The method of claim 1 wherein said polynucleotide is DNA.
7. The method of claim 1 wherein said polynucleotide is RNA.
8. The method of claim 1 wherein one of said first and second **oligonucleotide** probes is labeled with a sensitizer.
9. The method of claim 1 wherein one of said first and second **oligonucleotide** probes is labeled with a chemiluminescent compound.
10. The method of claim 1 wherein said predetermined amount of said first **oligonucleotide** probe is about 50-fold less than the expected amount of said amplified target polynucleotide.
11. A method for detecting the amount of a target polynucleotide in a sample, said method comprising: (a) providing in single reaction medium (i) a sample suspected of containing said target polynucleotide, said target polynucleotide being in single stranded form, (ii) predetermined amounts of one or more reference polynucleotides, each of said reference polynucleotides comprising a first sequence that is common with a first sequence of said target polynucleotide and a second sequence that is different from a second sequence of said target polynucleotide, (iii) a predetermined amount of a first **oligonucleotide** probe that has a sequence that hybridizes with said sequence that is common wherein said first **oligonucleotide** probe has, or is capable of having, a sensitizer attached thereto, (iv) a second **oligonucleotide** probe that has a sequence that hybridizes only with said second sequence of said target polynucleotide wherein said second **oligonucleotide** probe has, or is capable of having, a first chemiluminescent compound attached thereto, and (v) one or more third **oligonucleotide** probes, each having a sequence that hybridizes only with a respective second sequence of one of said reference polynucleotide wherein each of said third **oligonucleotide** probes has, or is capable of having, a second chemiluminescent compound attached thereto, said first and said second chemiluminescent compounds differ in signal produced when activated by said photosensitizer; said second chemiluminescent compound being different for each of said third **oligonucleotide** probes, (b) subjecting said single reaction medium to isothermal conditions for amplifying with equal efficiency said target polynucleotide and each of said reference polynucleotide, wherein said first probe is not extended, and wherein said conditions permit formation of a substantially non-dissociative first termolecular complex of said target polynucleotide, said first **oligonucleotide** probe and said second **oligonucleotide** probe and a substantially non-dissociative second termolecular complex of each of said reference polynucleotides with said first **oligonucleotide** probe and a respective third **oligonucleotide** probe and wherein said predetermined amount of said first **oligonucleotide** probe is less than the expected amount of said amplified target polynucleotide, (c) determining the ratio of the amount of said signal produced by said first termolecular complex to the amount of signal produced by each of said second termolecular complexes, and (d) relating each of said ratios to the amount of each respective reference polynucleotide to determine the amount of said target polynucleotide in said sample.
12. The method of claim 11 wherein said chemiluminescent compounds are each independently selected from the group consisting of enol ethers, enamines, 9-alkylidene-N-alkylacridans, arylvinylethers, dioxenes, arylimidazoles, 9-alkylidene-xanthenes and lucigenin.
13. The method of claim 11 wherein said sensitizer is a photosensitizer.
14. The method of claim 13 wherein said photosensitizer is selected from the group consisting of methylene blue, rose bengal, porphyrins and phthalocyanines.
15. The method of claim 11 wherein said amplification is selected from the group consisting of NASBA, 3SR, SDA and amplifications utilizing Q β -replicase.
16. The method of claim 11 wherein said polynucleotide is DNA.
17. The method of claim 11 wherein said polynucleotide is RNA.

L5 ANSWER 81 OF 112 USPATFULL on STN

2002:60975 Avian and reptile derived polynucleotide encoding a polypeptide having heparanase activity.
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Pecker, Iris, Rishon LeZion, ISRAEL

Michal, Israel, Ashkelon, ISRAEL
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US 2002034810 A1 20020321

APPLICATION: US 2001-930218 A1 20010816 (9)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Avian and reptile derived heparanase and nucleic acids encoding same.

CLM What is claimed is:

1. An isolated nucleic acid comprising a genomic, complementary or composite polynucleotide sequence encoding a polypeptide being at least 75% similar to SEQ ID NO:4 or a portion thereof as determined using the BestFit software of the Wisconsin sequence analysis package, utilizing the Smith and Waterman algorithm, where gap weight equals 8 and length weight equals 2, average match equals 2.912 and average mismatch equals -2.003.

2. The isolated nucleic acid of claim 1, wherein said polypeptide has heparanase catalytic activity or said polypeptide is cleavable by a protease so as to have said heparanase catalytic activity.

3. An isolated nucleic acid comprising a genomic, complementary or composite polynucleotide sequence being at least 65% identical to SEQ ID NO:10 or a portion thereof as determined using the BestFit software of the Wisconsin sequence analysis package, utilizing the Smith and Waterman algorithm, where gap weight equals 50, length weight equals 3, average match equals 10 and average mismatch equals -9.

4. The isolated nucleic acid of claim 3, wherein said polynucleotide encodes a polypeptide which has heparanase catalytic activity or which is cleavable by a protease so as to have said heparanase catalytic activity.

5. An isolate nucleic acid as set forth in SEQ ID NO:10 or a portion thereof.

6. An isolated nucleic acid comprising a genomic, complementary or composite polynucleotide sequence being hybridizable with SEQ ID NO:10 or a portion thereof under hybridization conditions of hybridization solution containing 10% dextrane sulfate, 1 M NaCl, 1% SDS and 5×10^6 cpm ^{32}P labeled probe, at 65° C., with a final wash solution of 1×SSC and 0.1% SDS and final wash at 65° C.

7. The isolated nucleic acid of claim 6, wherein said polynucleotide encodes a polypeptide which has heparanase catalytic activity or which is cleavable by a protease so as to have said heparanase catalytic activity.

8. A nucleic acid construct comprising the isolated nucleic acid of claim 1.

9. A nucleic acid construct comprising the isolated nucleic acid of claim 3.

10. A nucleic acid construct comprising the isolated nucleic acid of claim 4.

11. A nucleic acid construct comprising the isolated nucleic acid of claim 6.

12. A cell transformed or transfected with the nucleic acid of claim 1.

13. A cell transformed or transfected with the nucleic acid of claim 3.

14. A cell transformed or transfected with the nucleic acid of claim 4.

15. A cell transformed or transfected with the nucleic acid of claim 6.

16. An **oligonucleotide** of at least 17 bases specifically hybridizable with the isolated nucleic acid of claim 3 and which is not hybridizable with any mammalian heparanase cDNA.

17. A pair of **oligonucleotides** each of at least 17 bases specifically hybridizable with the isolated nucleic acid of claim 3 in an opposite orientation so as to direct exponential amplification of a portion thereof in a nucleic acid amplification reaction, and which are not hybridizable with any mammalian heparanase cDNA..

18. A nucleic acid amplification product obtained using the pair of **primers** of claim 17.

similar to SEQ ID NO:4 or a portion thereof as determined using the BestFit software of the Wisconsin sequence analysis package, utilizing the Smith and Waterman algorithm, where gap weight equals 8 and length weight equals 2, average match equals 2.912 and average mismatch equals -2.003.

20. The recombinant protein of claim 19, wherein said polypeptide has heparanase catalytic activity or said polypeptide is cleavable by a protease so as to have said heparanase catalytic activity.

21. A recombinant protein comprising a polypeptide being encoded by a nucleic acid including a genomic, complementary or composite polynucleotide sequence being at least 65% identical to SEQ ID NO:10 or a portion thereof as determined using the BestFit software of the Wisconsin sequence analysis package, utilizing the Smith and Waterman algorithm, where gap weight equals 50, length weight equals 3, average match equals 10 and average mismatch equals -9.

22. The recombinant protein of claim 21, wherein said polypeptide has heparanase catalytic activity or said polypeptide is cleavable by a protease so as to have said heparanase catalytic activity.

23. A recombinant protein comprising a polypeptide being encoded by a nucleic acid as set forth in SEQ ID NO:10 or a portion thereof.

24. A recombinant protein comprising a polypeptide being encoded by a nucleic acid including a genomic, complementary or composite polynucleotide sequence being hybridizable with SEQ ID NO:10 or a portion thereof under hybridization conditions of hybridization solution containing 10% dextrane sulfate, 1 M NaCl, 1% SDS and 5×10^6 cpm 32 P labeled probe, at 65° C., with a final wash solution of 1×SSC and 0.1% SDS and final wash at 65° C.

25. The recombinant protein of claim 24, wherein said polypeptide has heparanase catalytic activity or said polypeptide is cleavable by a protease so as to have said heparanase catalytic activity.

26. A pharmaceutical composition comprising, as an active ingredient, the recombinant protein of claim 19 and a pharmaceutically acceptable carrier.

27. A pharmaceutical composition comprising, as an active ingredient, the recombinant protein of claim 21 and a pharmaceutically acceptable carrier.

28. A pharmaceutical composition comprising, as an active ingredient, the recombinant protein of claim 23 and a pharmaceutically acceptable carrier.

29. A pharmaceutical composition comprising, as an active ingredient, the recombinant protein of claim 24 and a pharmaceutically acceptable carrier.

30. A medical equipment comprising a medical device containing, as an active ingredient, a recombinant protein having heparanase catalytic activity.

31. A nucleic acid construct comprising a first polynucleotide encoding a peptide as set forth at positions 1 to 19 of SEQ ID NO:4 and an in frame, second polynucleotide encoding a membrane targeted or secreted polypeptide.

32. The nucleic acid construct of claim 31, wherein said membrane targeted or secreted polypeptide is human heparanase.

33. A nucleic acid construct comprising a first polynucleotide encoding a signal peptide of avian or reptile heparanase and an in frame, second polynucleotide encoding a membrane targeted or secreted polypeptide.

34. The nucleic acid construct of claim 33, wherein said membrane targeted or secreted polypeptide is human heparanase.

35. A method of expressing a protein of interest in a cell, the method comprising: transforming the cell with a nucleic acid construct that comprises a first polynucleotide encoding a signal peptide of avian or reptile heparanase and an in frame, second polynucleotide encoding a membrane targeted or secreted polypeptide; and culturing the cell.

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Millennium Pharmaceuticals, Inc. (U.S. corporation)
US 2002034780 A1 20020321

APPLICATION: US 2001-799875 A1 20010306 (9)

PRIORITY: US 2000-182059P 20000211 (60)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention relates to novel kinase nucleic acid sequences and proteins. Also provided are vectors, host cells, and recombinant methods for making and using the novel molecules.

CLM What is claimed is:

1. An isolated nucleic acid molecule selected from the group consisting of: a) a nucleic acid molecule comprising a nucleotide sequence having at least 60% sequence identity to the nucleotide sequence of SEQ ID NO:4, 6, 7, 9, 10, 12, 13, 15, 16, or 18, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-2204, PTA-1868, _____, _____, or _____, wherein said sequence encodes a polypeptide having biological activity; b) a nucleic acid molecule comprising a fragment of at least 20 nucleotides of the nucleotide sequence of SEQ ID NO: 4, 6, 7, 9, 10, 12, 13, 15, 16, or 18, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-2204, PTA-1868, _____, _____, or _____; c) a nucleic acid molecule which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:5, 8, 11, 14 or 17, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number PTA-2204, PTA-1868, _____, _____, or _____; d) a nucleic acid molecule which encodes a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:5, 8, 11, 14, or 17, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number PTA-2204, PTA-1868, _____, _____, or _____, wherein the fragment comprises at least 15 contiguous amino acids of SEQ ID NO: 5, 8, 11, 14, or 17, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number PTA-2204, PTA-1868, _____, _____, or _____; e) a nucleic acid molecule which encodes a naturally occurring allelic variant of a biologically active polypeptide comprising the amino acid sequence of SEQ ID NO: 5, 8, 11, 14, or 17, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number PTA-2204, PTA-1868, _____, _____, or _____, wherein the nucleic acid molecule hybridizes to a nucleic acid molecule comprising the complement of SEQ ID NO: 4, 6, 7, 9, 10, 12, 13, 15, 16, or 18 under stringent conditions; and, f) a nucleic acid molecule comprising the complement of a), b), c), d), or e).

2. The isolated nucleic acid molecule of claim 1, which is selected from the group consisting of: a) a nucleic acid comprising the nucleotide sequence of SEQ ID NO: 4, 6, 7, 9, 10, 12, 13, 15, 16, or 18, the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-2204, PTA-1868, _____, _____, or _____, or complement thereof; and, b) a nucleic acid molecule which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:5, 8, 11, 14 or 17, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number PTA-2204, PTA-1868, _____, _____, or _____.

3. The nucleic acid molecule of claim 1 further comprising vector nucleic acid sequences.

4. The nucleic acid molecule of claim 1 further comprising nucleic acid sequences encoding a heterologous polypeptide.

5. A host cell which contains the nucleic acid molecule of claim 1.

6. The host cell of claim 5 which is a mammalian host cell.

7. A non-human mammalian host cell containing the nucleic acid molecule of claim 1.

8. An isolated polypeptide selected from the group consisting of: a) a biologically active polypeptide which is encoded by a nucleic acid molecule comprising a nucleotide sequence having at least 60% sequence identity to a nucleic acid comprising the nucleotide sequence of SEQ ID NO: 4, 6, 7, 9, 10, 12, 13, 15, 16, or 18, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-2204, PTA-1868, _____, _____, or _____; b) a naturally occurring allelic variant of a biologically active polypeptide comprising the amino acid sequence of SEQ ID NO: 5, 8, 11, 14 or 17, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number PTA-2204, PTA-1868, _____, _____, or _____, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a nucleic acid molecule comprising the complement of SEQ

and, c) a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO: 5, 8, 11, 14, or 17, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number PTA-2204, PTA-1868, _____, _____, or _____, wherein the fragment comprises at least 15 contiguous amino acids of SEQ ID NO: 5, 8, 11, 14, or 17; and, d) a biologically active polypeptide having at least 60% sequence identity to the amino acid sequence SEQ ID NO: 5, 8, 11, 14, or 17.

9. The isolated polypeptide of claim 8 comprising the amino acid sequence of SEQ ID NO: 5, 8, 11, 14, or 17.

10. The polypeptide of claim 8 further comprising heterologous amino acid sequences.

11. An antibody which selectively binds to a polypeptide of claim 8.

12. A method for producing a polypeptide selected from the group consisting of: a) a polypeptide comprising the amino acid sequence of SEQ ID NO: 5, 8, 11, 14 or 17, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number PTA-2204, PTA-1868, _____, _____, or _____; b) a polypeptide comprising a fragment of the amino acid sequence of SEQ ID NO: 5, 8, 11, 14 or 17, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number PTA-2204, PTA-1868, _____, _____, or _____, wherein the fragment comprises at least 15 contiguous amino acids of SEQ ID NO: 5, 8, 11, 14, or 17, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number PTA-2204, PTA-1868, _____, _____, or _____; c) a naturally occurring allelic variant of a biologically active polypeptide comprising the amino acid sequence of SEQ ID NO: 5, 8, 11, 14 or 17, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number PTA-2204, PTA-1868, _____, _____, or _____, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a nucleic acid molecule comprising the complement of SEQ ID NO: 4, 6, 7, 9, 10, 12, 13, 15, 16, or 18; and, d) a biologically active polypeptide having at least 60% sequence identity to the nucleic acid sequence of SEQ ID NO: 4, 6, 7, 9, 10, 12, 13, 15, 16 or 18; comprising culturing a host cell under conditions in which the nucleic acid molecule is expressed.

13. A method for detecting the presence of a polypeptide of claim 8 in a sample, comprising: a) contacting the sample with a compound which selectively binds to a polypeptide of claim 8; and b) determining whether the compound binds to the polypeptide in the sample.

14. The method of claim 13, wherein the compound which binds to the polypeptide is an antibody.

15. A kit comprising a compound which selectively binds to a polypeptide of claim 8 and instructions for use.

16. A method for detecting the presence of a nucleic acid molecule of claim 1 in a sample, comprising the steps of: a) contacting the sample with a nucleic acid probe or **primer** which selectively hybridizes to the nucleic acid molecule; and b) determining whether the nucleic acid probe or **primer** binds to a nucleic acid molecule in the sample.

17. The method of claim 16, wherein the sample comprises mRNA molecules and is contacted with a nucleic acid probe.

18. A kit comprising a compound which selectively hybridizes to a nucleic acid molecule of claim 1 and instructions for use.

19. A method for identifying a compound which binds to a polypeptide of claim 8 comprising the steps of: a) contacting a polypeptide, or a cell expressing a polypeptide of claim 8 with a test compound; and b) determining whether the polypeptide binds to the test compound.

20. The method of claim 19, wherein the binding of the test compound to the polypeptide is detected by a method selected from the group consisting of: a) detection of binding by direct detecting of test compound/polypeptide binding; b) detection of binding using a competition binding assay; c) detection of binding using an assay for a kinase-like activity.

21. A method for modulating the activity of a polypeptide of claim 8 comprising contacting a polypeptide or a cell expressing a polypeptide of claim 8 with a compound which binds to the polypeptide in a sufficient concentration to modulate the activity of the polypeptide.

22. A method for identifying a compound which modulates the activity of

claim 8 with a test compound; and b) determining the effect of the test compound on the activity of the polypeptide to thereby identify a compound that modulates the activity of the polypeptide.

L5 ANSWER 83 OF 112 USPTAFULL on STN

2002:22084 Nucleic acids of rochalimaea henselae and methods and compositions for diagnosing rochalimaea henselae and rochalimaea quintana infection.

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Regnery, Russell L., Tucker, GA, UNITED STATES

The Government of the United States, Department of Health and Human Services (U.S. corporation)

US 2002012919 A1 20020131

APPLICATION: US 2000-752385 A1 20001229 (9)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method of diagnosing cat scratch disease and a method of diagnosing bacillary angiomatosis in a subject by detecting the presence of Rochalimaea henselae or an antigenic fragment thereof in the subject is provided. Also provided is a vaccine comprising an immunogenic amount of a nonpathogenic Rochalimaea henselae or an immunogenically specific determinant thereof and a pharmaceutically acceptable carrier. A method of diagnosing Rochalimaea quintana infection in a subject by detecting the presence of a nucleic acid specific to Rochalimaea quintana in a sample from the subject is provided. A purified, 60-kDa heat shock protein of Rochalimaea is provided. Also provided is a 17-kDa antigenic polypeptide of Rochalimaea.

CLM What is claimed is:

1. An isolated nucleic acid encoding the amino acid sequence set forth in the Sequence Listing as SEQ ID NO:11.
2. The isolated nucleic acid of claim 1 in a vector suitable for expressing the nucleic acid.
3. The vector of claim 2 in a host suitable for expressing the nucleic acid.
4. An isolated nucleic acid, of at least 15 nucleotides in length, which can selectively hybridize with the nucleic acid of claim 1, under **polymerase chain reaction** conditions.
5. An isolated nucleic acid, of at least 15 nucleotides in length, which can specifically hybridize with the nucleic acid of claim 1, under the stringency conditions of 60° C. and 5×SSC, followed by the initial washing condition of room temperature, 2×SSC and 0.1% SDS, and two secondary washes with stringency conditions of 50° C., 0.5% SSC and 0.1% SDS.
6. A purified antigenic polypeptide encoded by the isolated nucleic acid of claim 5.
7. A purified antigenic polypeptide having the amino acid sequence set forth in the Sequence Listing as SEQ ID NO:11.
8. A method of diagnosing current or previous cat scratch disease in a subject, comprising detecting the presence of Rochalimaea henselae in a sample from the subject, and correlating the presence of Rochalimaea henselae with cat scratch disease in the subject.
9. The method of claim 8, wherein the detecting step comprises the steps of contacting a fluid or tissue sample from the subject with a detectable amount of a purified antibody that specifically binds Rochalimaea henselae, and detecting the binding of the antibody to Rochalimaea henselae.
10. The method of claim 9, wherein the antibody is an antibody which specifically binds to the polypeptide set forth in the Sequence Listing as SEQ ID NO:11.
11. The method of claim 9, wherein the antibody is an antibody which specifically binds to the polypeptide set forth in the Sequence Listing as SEQ ID NO:7.
12. A method of diagnosing current or previous cat scratch disease in a subject by detecting the presence of an antibody that specifically binds R. henselae, wherein the detecting step comprises the steps of contacting an antibody-containing fluid or tissue sample from the subject with an amount of purified Rochalimaea henselae or an antigenic fragment thereof which binds to the antibody, detecting the binding of the Rochalimaea henselae or antigenic fragment thereof to the antibody, and correlating the presence of an antibody that specifically binds to Rochalimaea henselae or an antigenic fragment thereof with cat scratch

13. The method of claim 12, wherein the antigenic fragment is the antigenic polypeptide encoded by the nucleic acid which can specifically hybridize with the nucleic acid encoding the amino acid sequence set forth in the Sequence Listing as SEQ ID NO:11 under the stringency conditions of 60° C. and 5×SSC, followed by the initial washing condition of room temperature, 2×SSC and 0.1% SDS, and two secondary washes with stringency conditions of 50° C., 0.5% SSC and 0.1% SDS.

14. The method of claim 12, wherein the antigenic fragment is the polypeptide set forth in the Sequence Listing as SEQ ID NO:11.

15. The method of claim 8, wherein the presence of *Rochalimaea henselae* is determined by detecting the presence of a nucleic acid specific for *Rochalimaea henselae*.

16. The method of claim 15, wherein the nucleic acid detected is a nucleic acid encoding the amino acid sequence set forth in the Sequence Listing as SEQ ID NO:11.

17. The method of claim 15, wherein the nucleic acid is detected utilizing a nucleic acid amplification technique.

18. The method of claim 8, wherein the subject is a human.

19. A method of diagnosing bacillary angiomatosis in a subject comprising detecting the presence of *Rochalimaea henselae* in a sample from the subject, and correlating the presence of *Rochalimaea henselae* with bacillary angiomatosis in the subject.

20. The method of claim 19, wherein the detecting step comprises the steps of contacting a fluid or tissue sample from the subject with a detectable amount of a purified antibody that specifically binds *Rochalimaea henselae*, and detecting the binding of the antibody to *Rochalimaea henselae*.

21. A method of diagnosing bacillary angiomatosis in a subject by detecting the presence of an antibody that specifically binds *Rochalimaea henselae*, wherein the detecting step comprises the steps of contacting an antibody-containing fluid or tissue sample from the subject with an amount of purified *Rochalimaea henselae* or antigenic fragment thereof which binds to the antibody, and detecting the binding of the *Rochalimaea henselae* or antigenic fragment thereof to the antibody.

22. The method of claim 19, wherein the presence of *Rochalimaea henselae* is determined by detecting the presence of a nucleic acid specific for *Rochalimaea henselae*.

23. The method of claim 19, wherein the subject is a human.

24. A nonpathogenic *Rochalimaea henselae* or an immunogenic fragment thereof.

25. A purified *Rochalimaea henselae* bound to a ligand.

26. The purified *Rochalimaea henselae* of claim 25, wherein the ligand is an antibody.

27. A purified *Rochalimaea henselae* bound to a substrate.

28. A purified antibody or antigen binding fragment thereof that specifically binds *Rochalimaea henselae*.

29. The antibody of claim 28, wherein the antibody is a monoclonal antibody.

30. The antibody of claim 28, bound to a substrate.

31. The antibody of claim 28, wherein the antibody is labeled with a detectable moiety.

32. The antibody of claim 31, wherein the detectable moiety is fluorescent.

33. A diagnostic kit for detecting the presence of a primary antibody specifically reactive with *Rochalimaea henselae* or an immunogenic fragment thereof comprising: a purified *Rochalimaea henselae* or immunogenic fragment thereof bound to a substrate; a secondary antibody reactive with the antibody specifically reactive with *Rochalimaea henselae* or an immunogenic fragment thereof; and a reagent for detecting

34. An isolated immunogenically specific determinant of *Rochalimaea henselae*.
35. The method of claim 12, wherein the antigenic fragment is the polypeptide set forth in the Sequence Listing as SEQ ID NO:7.
36. The method of claim 15, wherein the nucleic acid specific for *Rochalimaea henselae* comprises the nucleotides in the nucleotide sequence defined in the Sequence Listing as SEQ ID NO:5.
37. The method of claim 22, wherein the nucleic acid specific for *Rochalimaea henselae* comprises the nucleotides in the nucleotide sequence defined in the Sequence Listing as SEQ ID NO:5.
38. An isolated nucleic acid specific for *Rochalimaea henselae*, comprising the nucleotides in the nucleotide sequence defined in the Sequence Listing as SEQ ID NO:5.
39. An isolated nucleic acid consisting of the nucleotides in the nucleotide sequence defined in the Sequence Listing as SEQ ID NO:7.
40. The nucleic acid of claim 39 in a vector suitable for expressing the nucleic acid.
41. The vector of claim 40 in a host suitable for expressing the nucleic acid.
42. A purified antigenic protein encoded by the nucleic acid of claim 39.
43. An isolated nucleic acid that specifically hybridizes with the nucleic acid of claim 39 under high stringency conditions and at least 85% sequence complementarity with the segment of the strand to which it hybridizes.
44. An antigenic polypeptide fragment encoded by the nucleic acid of claim 39.
45. A method of diagnosing bacillary angiomatosis in a subject comprising detecting the presence of *Rochalimaea quintana* in a sample from the subject by detecting the presence of a nucleic acid specific for *Rochalimaea quintana*, wherein the nucleic acid specific for *Rochalimaea quintana* consists of the nucleotides in the nucleotide sequence defined in the Sequence Listing as SEQ ID NO:6, and correlating the presence of *Rochalimaea quintana* with the presence of bacillary angiomatosis in the subject.
46. A method of diagnosing bacillary angiomatosis in a subject comprising detecting the presence of *Rochalimaea quintana* in a sample from the subject by detecting the presence of a nucleic acid specific for *Rochalimaea quintana*, wherein the nucleic acid specific for *Rochalimaea quintana* consists of the nucleotides in the nucleotide sequence defined in the Sequence Listing as SEQ ID NO:1, and correlating the presence of *Rochalimaea quintana* with the presence of bacillary angiomatosis in the subject.
47. An isolated nucleic acid specific for *Rochalimaea quintana*, comprising the nucleotides in the nucleotide sequence defined in the Sequence Listing as SEQ ID NO:6.
48. A method of diagnosing cat scratch disease or bacillary angiomatosis in a subject, comprising: amplifying DNA from the subject using a **primer** mixture consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:4; contacting the amplified DNA from step a. with a probe consisting of the nucleic acid of SEQ ID NO:5 and detecting the hybridization of the probe with the amplified DNA, the existence of hybridization indicating the presence of *R. henselae*, which is correlated with cat scratch disease; and contacting the amplified DNA from step a. with a probe consisting of the nucleic acid of SEQ ID NO:6 and detecting the hybridization of the probe with the amplified DNA, the existence of hybridization indicating the presence of *R. quintana*, which is correlated with bacillary angiomatosis.
49. A mixture of the nucleic acids of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:4.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to methods for using a human protein kinase belonging to the superfamily of mammalian protein kinases. The invention also relates to methods for using polynucleotides encoding the protein kinase. The invention relates to methods using the protein kinase polypeptides and polynucleotides as a target for diagnosis and treatment in protein kinase-mediated or -related disorders. The invention further relates to drug-screening methods using the protein kinase polypeptides and polynucleotides to identify agonists and antagonists for diagnosis and treatment. The invention further encompasses agonists and antagonists based on the protein kinase polypeptides and polynucleotides. The invention further relates to agonists and antagonists identified by drug screening methods with the protein kinase polypeptides and polynucleotides as a target.

CLM What is claimed is:

1. A method of identifying an agent that binds a protein kinase, said method comprising combining an agent to be tested with a host cell expressing a nucleotide sequence selected from the group consisting of: a) the nucleotide sequence corresponding to nucleotides 1381 to 3366 of SEQ ID NO:1; b) the nucleotide sequence set forth in SEQ ID NO:3; c) a nucleotide sequence encoding the amino acid sequence set forth in SEQ ID NO:2; d) a nucleotide sequence corresponding to the cDNA insert of the plasmid deposited with ATCC as Patent Deposit No. PTA-2201; e) a nucleotide sequence that is at least 60% identical to nucleotides 1381 to 3366 of SEQ ID NO:1; f) a nucleotide sequence that is at least 60% identical to the nucleotide sequence set forth in SEQ ID NO:3; and, g) a nucleotide sequence that is at least 60% identical to the nucleotide sequence corresponding to the cDNA insert of the plasmid deposited with ATCC as Patent Deposit No. PTA-2201; under conditions suitable for binding, and detecting the formation of a complex between said agent and said protein kinase; wherein said host cell is selected from the group consisting of brain, skeletal muscle, heart, fetal kidney; fetal heart; osteoblast; a virus-infected cell; vascular endothelium; vascular smooth muscle, and cells involved in tissue fibrosis.

2. The method of claim 1, wherein said method is a competition assay, in which binding is determined in the presence of one or more agents.

3. A method of identifying a compound that inhibits binding of an agent to a protein kinase said method comprising combining a compound to be tested and said agent with a host cell expressing a nucleotide sequence selected from the group consisting of: a) the nucleotide sequence corresponding to nucleotides 1381 to 3366 of SEQ ID NO:1; b) the nucleotide sequence set forth in SEQ ID NO:3; c) a nucleotide sequence encoding the amino acid sequence set forth in SEQ ID NO:2; d) a nucleotide sequence corresponding to the cDNA insert of the plasmid deposited with ATCC as Patent Deposit No. PTA-2201; e) a nucleotide sequence that is at least 60% identical to nucleotides 1381 to 3366 of SEQ ID NO: 1; f) a nucleotide sequence that is at least 60% identical to the nucleotide sequence set forth in SEQ ID NO:3; and, g) a nucleotide sequence that is at least 60% identical to the nucleotide sequence corresponding to the cDNA insert of the plasmid deposited with ATCC as Patent Deposit No. PTA-2201; under conditions suitable for binding of said agent thereto, and detecting the formation of a complex between said protein kinase and said agent, whereby inhibition of complex formation by said compound is indicative that said compound inhibits binding of said agent to said protein kinase; wherein said host cell is selected from the group consisting of brain, skeletal muscle, heart, fetal kidney; fetal heart; osteoblast; a virus-infected cell; vascular endothelium; vascular smooth muscle, and cells involved in tissue fibrosis.

4. The method of claim 3, wherein said compound is an antibody or antibody fragment.

5. A method of identifying an inhibitor of a protein kinase said method comprising combining an agent to be tested with a host cell expressing a nucleotide sequence selected from the group consisting of: a) the nucleotide sequence corresponding to nucleotides 1381 to 3366 of SEQ ID NO:1; b) the nucleotide sequence set forth in SEQ ID NO:3; c) a nucleotide sequence encoding the amino acid sequence set forth in SEQ ID NO:2; d) a nucleotide sequence corresponding to the cDNA insert of the plasmid deposited with ATCC as Patent Deposit No. PTA-2201; e) a nucleotide sequence that is at least 60% identical to nucleotides 1381 to 3366 of SEQ ID NO:1; f) a nucleotide sequence that is at least 60% identical to the nucleotide sequence set forth in SEQ ID NO:3; and, g) a nucleotide sequence that is at least 60% identical to the nucleotide sequence corresponding to the cDNA insert of the plasmid deposited with

detecting a protein kinase activity, and assessing the ability of said agent to inhibit said protein kinase activity, whereby inhibition of said protein kinase activity by said agent is indicative that said agent is an inhibitor; wherein said host cell is selected from the group consisting of brain, skeletal muscle, heart, fetal kidney; fetal heart; osteoblast; a virus-infected cell; vascular endothelium; vascular smooth muscle, and cells involved in tissue fibrosis.

6. The method of claim 5, wherein said protein kinase activity is a signaling activity or a cellular response.

7. An inhibitor of a protein kinase identified according to the method of claim 5, wherein said inhibitor is an antagonist.

8. A method for detecting the presence of a polypeptide in a sample, said method comprising contacting said sample with an agent that specifically allows detection of the presence of the polypeptide in the sample and then detecting the presence of the polypeptide, wherein said polypeptide is selected from the group consisting of: a) a polypeptide having the amino acid sequence set forth in SEQ ID NO:2; b) a polypeptide encoded by the nucleotide sequence corresponding to nucleotides 1381 to 3366 of SEQ ID NO:1; c) a polypeptide encoded by the nucleotide sequence set forth in SEQ ID NO:3; d) a polypeptide encoded by a nucleotide sequence corresponding to the cDNA insert of the plasmid deposited with ATCC as Patent Deposit No. PTA-2201; e) a polypeptide encoded by a nucleotide sequence that is at least 60% identical to nucleotides 1381 to 3366 of SEQ ID NO:1; f) a polypeptide encoded by a nucleotide sequence that is at least 60% identical to the nucleotide sequence set forth in SEQ ID NO:3; g) a polypeptide encoded by a nucleotide sequence that is at least 60% identical to the nucleotide sequence corresponding to the cDNA insert of the plasmid deposited with ATCC as Patent Deposit No. PTA-2201; and, h) a fragment of any of the polypeptides of a)-g) wherein said fragment comprises at least 15 contiguous amino acids of SEQ ID NO:2; wherein said sample is derived from a cell selected from the group consisting of brain, skeletal muscle, heart, fetal kidney, fetal heart, osteoblast, vascular endothelium, vascular smooth muscle, a virus-infected cell, and a cell involved in tissue fibrosis.

9. A method for modulating the level or activity of a polypeptide, the method comprising contacting said polypeptide with an agent under conditions that allow the agent to modulate the level or activity of the polypeptide, wherein said polypeptide is selected from the group consisting of: a) a polypeptide having the amino acid sequence set forth in SEQ ID NO:2; b) a polypeptide encoded by the nucleotide sequence corresponding to nucleotides 1381 to 3366 of SEQ ID NO:1; c) a polypeptide encoded by the nucleotide sequence set forth in SEQ ID NO:3; d) a polypeptide encoded by a nucleotide sequence corresponding to the cDNA insert of the plasmid deposited with ATCC as Patent Deposit No. PTA-2201; e) a polypeptide encoded by a nucleotide sequence that is at least 60% identical to nucleotides 1381 to 3366 of SEQ ID NO:1; f) a polypeptide encoded by a nucleotide sequence that is at least 60% identical to the nucleotide sequence set forth in SEQ ID NO:3; g) a polypeptide encoded by a nucleotide sequence that is at least 60% identical to the nucleotide sequence corresponding to the cDNA insert of the plasmid deposited with ATCC as Patent Deposit No. PTA-2201; and, h) a fragment of any of the polypeptides of a)-g) wherein said fragment comprises at least 15 contiguous amino acids of SEQ ID NO:2; wherein said modulation occurs in cells selected from the group consisting of brain, skeletal muscle, heart, fetal kidney, fetal heart, osteoblast, vascular endothelium, vascular smooth muscle, a virus-infected cell, and a cell involved in tissue fibrosis.

10. A method for detecting the presence of a nucleic acid molecule in a sample, said method comprising contacting said sample with an agent that specifically allows detection of the presence of the nucleic acid molecule in the sample and then detecting the presence of the nucleic acid molecule, the nucleic acid molecule having a nucleotide sequence selected from the group consisting of: a) the nucleotide sequence corresponding to nucleotides 1381 to 3366 of SEQ ID NO:1; b) the nucleotide sequence set forth in SEQ ID NO:3; c) a nucleotide sequence encoding the amino acid sequence set forth in SEQ ID NO:2; d) a nucleotide sequence corresponding to the cDNA insert of the plasmid deposited with ATCC as Patent Deposit No. PTA-2201; e) a nucleotide sequence that is at least 60% identical to nucleotides 1381 to 3366 of SEQ ID NO:1; f) a nucleotide sequence that is at least 60% identical to the nucleotide sequence set forth in SEQ ID NO:3; and, g) a nucleotide sequence that is at least 60% identical to the nucleotide sequence corresponding to the cDNA insert of the plasmid deposited with ATCC as Patent Deposit No. PTA-2201; wherein said sample is derived from a cell selected from the group consisting of brain, skeletal muscle, heart, fetal kidney, fetal heart, osteoblast, vascular endothelium, vascular

fibrosis.

11. The method of claim 10, wherein the method comprises contacting the sample with an **oligonucleotide** that hybridizes under stringent conditions to a nucleotide sequence selected from the group consisting of: a) the nucleotide sequence corresponding to nucleotides 1381 to 3366 of SEQ ID NO:1; b) the nucleotide sequence set forth in SEQ ID NO:3; c) a nucleotide sequence encoding the amino acid sequence set forth in SEQ ID NO:2; d) a nucleotide sequence corresponding to the cDNA insert of the plasmid deposited with ATCC as Patent Deposit No. PTA-2201; e) a nucleotide sequence that is at least 60% identical to nucleotides 1381 to 3366 of SEQ ID NO: 1; f) a nucleotide sequence that is at least 60% identical to the nucleotide sequence set forth in SEQ ID NO:3; and, g) a nucleotide sequence that is at least 60% identical to the nucleotide sequence corresponding to the cDNA insert of the plasmid deposited with ATCC as Patent Deposit No. PTA-2201; and determining whether the **oligonucleotide** binds to the nucleic acid sequence in the sample.

12. The method of claim 11, wherein the nucleic acid whose presence is detected is mRNA.

13. A kit comprising reagents used for the method of claim 11, wherein the reagents comprise a compound that hybridizes under stringent conditions.

14. The method of claim 11 wherein a fragment of the nucleic acid is contacted.

15. A method for modulating the level or activity of a nucleic acid molecule, said method comprising contacting said nucleic acid molecule with an agent under conditions that allow the agent to modulate the level or activity of the nucleic acid molecule, said nucleic acid molecule having a nucleotide sequence selected from the group consisting of: a) the nucleotide sequence corresponding to nucleotides 1381 to 3366 of SEQ ID NO:1; b) the nucleotide sequence set forth in SEQ ID NO:3; c) a nucleotide sequence encoding the amino acid sequence set forth in SEQ ID NO:2; d) a nucleotide sequence corresponding to the cDNA insert of the plasmid deposited with ATCC as Patent Deposit No. PTA-2201; e) a nucleotide sequence that is at least 60% identical to nucleotides 1381 to 3366 of SEQ ID NO:1; f) a nucleotide sequence that is at least 60% identical to the nucleotide sequence set forth in SEQ ID NO:3; and, g) a nucleotide sequence that is at least 60% identical to the nucleotide sequence corresponding to the cDNA insert of the plasmid deposited with ATCC as Patent Deposit No. PTA-2201; wherein said modulation is in a cell selected from the group consisting of brain, skeletal muscle, heart, fetal kidney, fetal heart, osteoblast, vascular endothelium, vascular smooth muscle, a virus-infected cell, and a cell involved in tissue fibrosis.

16. A method of modulating the activity of a polypeptide in a patient having a disorder selected from the group consisting of liver fibrosis, lung fibrosis, atherosclerosis, osteoporosis, osteopetrosis, cancer, diabetic blindness, psoriasis, age-related macular degeneration, viral infection, viral infection with hepatitis B virus, liver fibrosis resulting from hepatitis B virus infection, and disorders with abnormal angiogenesis, the method comprising administering to said patient a therapeutically effective amount of an agent that modulates the level or activity of a nucleotide sequence selected from the group consisting of: a) the nucleotide sequence corresponding to nucleotides 1381 to 3366 of SEQ ID NO:1; b) the nucleotide sequence set forth in SEQ ID NO:3; c) a nucleotide sequence encoding the amino acid sequence set forth in SEQ ID NO:2; d) a nucleotide sequence corresponding to the cDNA insert of the plasmid deposited with ATCC as Patent Deposit No. PTA-2201; e) a nucleotide sequence that is at least 60% identical to nucleotides 1381 to 3366 of SEQ ID NO:1; f) a nucleotide sequence that is at least 60% identical to the nucleotide sequence set forth in SEQ ID NO:3; and, g) a nucleotide sequence that is at least 60% identical to the nucleotide sequence corresponding to the cDNA insert of the plasmid deposited with ATCC as Patent Deposit No. PTA-2201.

17. The method of claim 16 wherein said disorder is liver fibrosis.

18. A method of modulating the activity of a polypeptide in a patient having a disorder selected from the group consisting of liver fibrosis, lung fibrosis, atherosclerosis, osteoporosis, osteopetrosis, cancer, diabetic blindness, psoriasis, age-related macular degeneration, viral infection, viral infection with hepatitis B virus, liver fibrosis resulting from hepatitis B virus infection, and disorders with abnormal angiogenesis, the method comprising administering to a subject in need of treatment a therapeutically effective amount of an agent that modulates the level or activity of said polypeptide wherein said

having the amino acid sequence set forth in SEQ ID NO:2; b) a polypeptide encoded by the nucleotide sequence corresponding to nucleotides 1381 to 3366 of SEQ ID NO:1; c) a polypeptide encoded by the nucleotide sequence set forth in SEQ ID NO:3; d) a polypeptide encoded by a nucleotide sequence corresponding to the cDNA insert of the plasmid deposited with ATCC as Patent Deposit No. PTA-2201; e) a polypeptide encoded by a nucleotide sequence that is at least 60% identical to nucleotides 1381 to 3366 of SEQ ID NO:1; f) a polypeptide encoded by a nucleotide sequence that is at least 60% identical to the nucleotide sequence set forth in SEQ ID NO:3; g) a polypeptide encoded by a nucleotide sequence that is at least 60% identical to the nucleotide sequence corresponding to the cDNA insert of the plasmid deposited with ATCC as Patent Deposit No. PTA-2201.

19. A method for detecting a propensity of a patient to develop a liver disorder, said method comprising obtaining a sample from said patient and contacting said sample with an agent that specifically allows detection of the presence of a nucleic acid molecule in the sample and then detecting the presence of the nucleic acid molecule, the nucleic acid molecule selected from the group consisting of: a) the nucleotide sequence corresponding to nucleotides 1381 to 3366 of SEQ ID NO:1; b) the nucleotide sequence set forth in SEQ ID NO:3; c) a nucleotide sequence encoding the amino acid sequence set forth in SEQ ID NO:2; d) a nucleotide sequence corresponding to the cDNA insert of the plasmid deposited with ATCC as Patent Deposit No. PTA-2201; e) a nucleotide sequence that is at least 60% identical to nucleotides 1381 to 3366 of SEQ ID NO:1; f) a nucleotide sequence that is at least 60% identical to the nucleotide sequence set forth in SEQ ID NO:3; and, g) a nucleotide sequence that is at least 60% identical to the nucleotide sequence corresponding to the cDNA insert of the plasmid deposited with ATCC as Patent Deposit No. PTA-2201; wherein said sample is derived from a patient with or at risk for liver disorders.

L5 ANSWER 85 OF 112 USPTAFULL on STN

2001:235084 Isothermal transcription based assay for the detection and genotyping of **dengue virus**.

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US 6333150 B1 20011225

APPLICATION: US 1999-374584 19990813 (9)

DOCUMENT TYPE: Utility; GRANTED.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB An isothermal transcription based amplification assay for **dengue virus** RNA uses primer combinations for sequences within the envelope gene or the 3' non-coding region of the virus and a probe. Probes may be specific for a serotype of **dengue virus**.

CLM What is claimed is:

1. A method for the detection of **dengue virus** RNA, comprising: a) obtaining a sample which may contain **dengue virus** RNA; b) performing an isothermal transcription based amplification on the sample with two **oligonucleotide primers**, a first **primer** which comprises at least 10 consecutive nucleotides of a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:6 and SEQ ID NO:7, and a second **primer** which comprises at least 10 consecutive nucleotides of a sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:8 and SEQ ID NO:9; and c) detecting the amplification product using a labeled probe, whereby hybridization of the probe to the amplification product indicates the presence of **dengue virus** RNA in the sample.

2. The method of claim 1, wherein the first **primer** further comprises an RNA polymerase promoter sequence covalently bonded to the 5' end thereof.

3. The method of claim 2, wherein the RNA polymerase promoter sequence is a T7 RNA polymerase promoter sequence.

4. The method of claim 1, wherein the sample comprises cells or virus and RNA is extracted from the cells or virus in the sample prior to step (b).

5. The method of claim 1, wherein the probe is selected from the group consisting of SEQ ID NO:5 and SEQ ID NO:10.

6. A method for the detection or genotyping of **dengue virus** RNA in a sample comprising: a) obtaining a sample which may contain **dengue virus** RNA; b) performing an isothermal transcription based amplification on the sample with two **primers**, a first **primer** which comprises at least 10 consecutive nucleotides of a sequence according to

consecutive nucleotides of a sequence according to SEQ ID NO:12; and c) detecting or genotyping the amplification product using one or more probes.

7. The method of claim 6, wherein the first **primer** further comprises an RNA polymerase promoter sequence covalently bonded to the 5' end thereof.

8. The method of claim 7, wherein the RNA polymerase promoter sequence is a T7 RNA polymerase promoter sequence.

9. The method of claim 6, wherein the sample comprises cells or virus and RNA is extracted from the cells or virus in the sample prior to step (b).

10. The method of claim 6, wherein the probe is selected from the group consisting of SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16 and SEQ ID NO:17, whereby hybridization of the amplification product with SEQ ID NO:13 indicates the virus RNA is type 1, hybridization of the amplification product with SEQ ID NO:14 indicates the virus RNA is type 2, hybridization of the amplification product with SEQ ID NO:15 indicates the virus RNA is type 3, hybridization of the amplification product with SEQ ID NO:16 indicates the virus RNA is type 4, and wherein hybridization of the amplification product with SEQ ID NO:17 indicates the presence of **dengue virus** RNA.

11. A kit for the detection or genotyping of **dengue virus** RNA in a sample, comprising two **oligonucleotide primers**, a first **primer** being about 15-26 nucleotides in length and comprising at least 10 consecutive nucleotides of a sequence according to SEQ ID NO:11, and a second **primer** being about 15-26 nucleotides in length and comprising at least 10 consecutive nucleotides of a sequence according to SEQ ID NO:12, and at least one probe.

12. A kit according to claim 11, wherein the at least one probe is selected from the group consisting of SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16 and SEQ ID NO:17.

13. A kit for the detection of **dengue virus** type 2 RNA in a sample, said kit comprising two **oligonucleotide primers**, a first **primer** being about 15-26 nucleotides in length and comprising at least 10 consecutive nucleotides of a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:6 and SEQ ID NO:7, and a second **primer** being about 15-26 nucleotides in length and comprising at least 10 consecutive nucleotides of a sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:8, and SEQ ID NO:9; and a probe.

14. A kit according to claim 13, wherein the probe is selected from the group consisting of SEQ ID NO:5, SEQ ID NO:10, and SEQ ID NO:23.

15. An **oligonucleotide** of about 15-26 nucleotides in length comprising at least 10 consecutive nucleotides of a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:10, and SEQ ID NO:23.

16. The **oligonucleotide** of claim 15, comprising at least 10 consecutive nucleotides of a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:4 and SEQ ID NO:6, and SEQ ID NO:9.

17. The **oligonucleotide** of claim 15, comprising at least 10 consecutive nucleotides of a sequence selected from SEQ ID NO:1 and SEQ ID NO:6, further comprising an RNA polymerase promoter sequence covalently bonded to the 5' end thereof.

18. The **oligonucleotide** of claim 17, wherein the RNA polymerase promoter sequence is the T7 RNA polymerase promoter sequence.

19. An **oligonucleotide** of about 15-26 nucleotides in length, comprising at least 10 consecutive nucleotides of a sequence selected from the group consisting of SEQ ID NO:11, SEQ ID NO:12, and SEQ ID NO:14.

20. The **oligonucleotide** of claim 19, comprising at least 10 consecutive nucleotides of a sequence according to SEQ ID NO:11 or SEQ ID NO:12.

21. The **oligonucleotide** of claim 19, comprising at least 10 consecutive nucleotides of a sequence according to SEQ ID NO:11, further comprising an RNA polymerase promoter sequences covalently bonded to the 5' end thereof.

promoter sequence is the T7 RNA polymerase promoter sequence.

23. The **oligonucleotide** of claim 22, wherein the **oligonucleotide** is a sequence according to SEQ ID NO:22.

24. An **oligonucleotide** of 15-26 nucleotides in length which is a subsequence of **Dengue virus** and which comprises at least 15 consecutive nucleotides of a sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:7, and SEQ ID NO:8, wherein said consecutive nucleotides are identical throughout their length to a **Dengue virus** genome.

25. An **oligonucleotide** of 15-26 nucleotides in length which is a subsequence of **Dengue virus** and which comprises at least 15 consecutive nucleotides of a sequence selected from the group consisting of SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17, wherein said consecutive nucleotides are identical throughout their length to a **Dengue virus** genome.

L5 ANSWER 86 OF 112 USPTAFULL on STN

2001:190901 Arrays of nucleic acid probes for analyzing biotransformation genes and methods of using the same.

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US 6309823 B1 20011030

APPLICATION: US 1997-778794 19970103 (8)

DOCUMENT TYPE: Utility; GRANTED.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention provides arrays of immobilized probes, and methods employing the arrays, for detecting mutations in the biotransformation genes, such as cytochromes P450. For example, one such array comprises four probe sets. A first probe set comprises a plurality of probes, each probe comprising a segment of at least three nucleotides exactly complementary to a subsequence of a reference sequence from a biotransformation gene, the segment including at least one interrogation position complementary to a corresponding nucleotide in the reference sequence. Second, third and fourth probe sets each comprise a corresponding probe for each probe in the first probe set. The probes in the second, third and fourth probe sets are identical to a sequence comprising the corresponding probe from the first probe set or a subsequence of at least three nucleotides thereof that includes the at least one interrogation position, except that the at least one interrogation position is occupied by a different nucleotide in each of the four corresponding probes from the four probe sets.

CLM What is claimed is:

1. An array of nucleic acid probes immobilized on a solid support, the array comprising at least two sets of probes, (1) a first probe set comprising a plurality of probes, each probe comprising a segment of at least six nucleotides exactly complementary to a subsequence of a reference sequence, the segment including at least one interrogation position complementary to a corresponding nucleotide in the reference sequence, (2) a second probe set comprising a corresponding probe for each probe in the first probe set, the corresponding probe in the second probe set being identical to a sequence comprising the corresponding probe from the first probe set or a subsequence of at least six nucleotides thereof that includes the at least one interrogation position, except that the at least one interrogation position is occupied by a different nucleotide in each of the two corresponding probes from the first and second probe sets; wherein the probes in the first probe set have at least three interrogation positions respectively corresponding to each of three contiguous nucleotides in the reference sequence; provided that the array does not consist of a complete set of probes of a given length, wherein a complete set is all permutations of nucleotides A, C, G and T/U; wherein the reference sequence is from a biotransformation gene.

2. An array of **oligonucleotide** probes immobilized on a solid support, the array comprising at least four sets of nucleic acid probes, (1) a first probe set comprising a plurality of probes, each probe comprising a segment of at least six nucleotides exactly complementary to a subsequence of a reference sequence, the segment including at least one interrogation position complementary to a corresponding nucleotide in

comprising a corresponding probe for each probe in the first probe set, the probes in the second, third and fourth probe sets being identical to a sequence comprising the corresponding probe from the first probe set or a subsequence of at least six nucleotides thereof that includes the at least one interrogation position, except that the at least one interrogation position is occupied by a different nucleotide in each of the four corresponding probes from the four probe sets; provided the array does not consist of a complete set of probes of a given length wherein a complete set is all permutations of nucleotides A, C, G and T/U; wherein the reference sequence is from a biotransformation gene.

3. The array of claim 1 wherein the reference sequence is from a gene encoding an enzyme selected from the group consisting of a cytochrome P450, N-acetyl transferase II, glucose 6-phosphate dehydrogenase, pseudocholinesterase, catechol-O-methyl transferase, and dihydropyridine dehydrogenase.

4. The array of claim 2, wherein the reference sequence is from a gene encoding an enzyme selected from the group consisting of a cytochrome P450, N-acetyl transferase II, glucose 6-phosphate dehydrogenase, pseudocholinesterase, catechol-O-methyl transferase, and dihydropyridine dehydrogenase.

5. The array of claim 4, wherein the enzyme is P450 2D6 or P450 2C19.

6. The array of claim 2, wherein the reference sequence includes a site of a mutation and a site of a silent polymorphism.

7. The array of claim 6, wherein the silent polymorphism is in an intron or flanking region of a gene.

8. The array of claim 2, wherein the first probe set has at least 3 interrogation positions respectively corresponding to each of 3 contiguous nucleotides in the reference sequence.

9. The array of claim 2, wherein the array has between 100 and 100,000 probes.

10. The array of claim 2, wherein the probes are linked to the support via a spacer.

11. The array of claim 2, wherein the segment in each probe of the first probe set that is exactly complementary to the subsequence of the reference sequence is 9-21 nucleotides.

12. An array of nucleic acid probes immobilized on a solid support, the array comprising at least one pair of first and second probe groups, each group comprising a first and second sets of **oligonucleotide** probes as defined by claim 1; wherein each probe in the first probe set from the first group is exactly complementary to a subsequence of a first reference sequence and each probe in the first probe set from the second group is exactly complementary to a subsequence from a second reference sequence.

13. The array of claim 12, wherein each group further comprises third and fourth probe sets, each comprising a corresponding probe for each probe in the first probe set, the probes in the second, third and fourth probe sets being identical to a sequence comprising the corresponding probe from the first probe set or a subsequence of at least six nucleotides thereof that includes the interrogation position, except that the interrogation position is occupied by a different nucleotide in each of the four corresponding probes from the four probe sets.

14. The array of claim 12, wherein the first reference sequence includes the site of a mutation in the biotransformation gene, and the second reference sequence includes a site of a silent polymorphism within the biotransformation gene or flanking the biotransformation gene.

15. The array of claim 14, wherein the reference sequence is from a gene encoding an enzyme selected from the group consisting of a cytochrome P450, N-acetyl transferase II, glucose 6-phosphate dehydrogenase, pseudocholinesterase, catechol-O-methyl transferase, and dihydropyridine dehydrogenase.

16. The array of claim 14 that comprises at least forty pairs of first and second probe groups, wherein the probes in the first probe sets from the first groups of the forty pairs are exactly complementary to subsequences from forty respective first reference sequences.

17. A block of nucleic acid probes immobilized on a solid support, comprising: a perfectly matched probe comprising a segment of at least six nucleotides exactly complementary to a subsequence of a reference

respectively corresponding to a plurality of nucleotides in the reference sequence, for each interrogation position, three mismatched probes, each identical to a sequence comprising the perfectly matched probe or a subsequence of at least six nucleotides thereof including the plurality of interrogation positions, except in the interrogation position, which is occupied by a different nucleotide in each of the three mismatched probes and the perfectly matched probe; provided the array does not consist of a complete set of probes of a given length, wherein a complete set is all permutations of nucleotides A, C, G and T/U; wherein the reference sequence is from a biotransformation gene.

18. The array of claim 16, wherein the segment of the perfectly matched probe comprises 3-20 interrogation positions corresponding to 3-20 respective nucleotides in the reference sequence.

19. An array of probes immobilized to a solid support comprising at least two blocks of probes, each block as defined by claim 16, a first block comprising a perfectly matched probe comprising a segment exactly complementary to a subsequence of a first reference sequence and a second block comprising a perfectly matched probe comprising a segment exactly complementary to a subsequence of a second reference sequence.

20. The array of claim 19, wherein the first reference sequence is from a wildtype 2D6 gene and the second reference sequence is from a mutant 2D6 gene.

21. The array of claim 19, comprising at least 10-100 blocks of probes, each comprising a perfectly matched probe comprising a segment exactly complementary to a subsequence of at least 10-100 respective reference sequences.

22. An array of nucleic acid probes immobilized on a solid support, the array comprising at least four probes: a first probe comprising first and second segments, each of at least three nucleotides and exactly complementary to first and second subsequences of a reference sequence, the segments including at least one interrogation position corresponding to a nucleotide in the reference sequence, wherein either (1) the first and second subsequences are noncontiguous, or (2) the first and second subsequences are contiguous and the first and second segments are inverted relative to the complement of the first and second subsequences in the reference sequence; second, third and fourth probes, identical to a sequence comprising the first probe or a subsequence thereof comprising at least three nucleotides from each of the first and second segments, except in the at least one interrogation position, which differs in each of the probes; provided the array does not consist of a complete set of probes of a given length, wherein a complete set is all permutations of nucleotides A, C, G and T/U; wherein the reference sequence is from a biotransformation gene.

23. A method of comparing a target nucleic acid with a reference sequence comprising a predetermined sequence of nucleotides, the method comprising: (a) hybridizing a sample comprising the target nucleic acid to an array of nucleic acid probes immobilized on a solid support, the array comprising: (1) a first probe set comprising a plurality of probes, each probe comprising a segment of at least six nucleotides exactly complementary to a subsequence of the reference sequence, the segment including at least one interrogation position complementary to a corresponding nucleotide in the reference sequence, wherein the reference sequence is from a biotransformation gene; (2) a second probe set comprising a corresponding probe for each probe in the first probe set, the corresponding probe in the second probe set being identical to a sequence comprising the corresponding probe from the first probe set or a subsequence of at least six nucleotides thereof that includes the at least one interrogation position, except that the at least one interrogation position is occupied by a different nucleotide in each of the two corresponding probes from the first and second probe sets; wherein, the probes in the first probe set have at least three interrogation positions respectively corresponding to each of at least three nucleotides in the reference sequence, and (b) comparing the relative specific hybridization of two corresponding probes from the first and second probe sets; (c) assigning a nucleotide in the target sequence as the complement of the interrogation position of the probe having the greater specific binding; (d) repeating (b) and (c) by comparing the relative specific hybridization of a further two corresponding probes from the first and second probe sets until each nucleotide of interest in the target sequence has been assigned.

24. The method of claim 23, wherein the determining step comprises: (1) comparing the relative specific hybridization of two corresponding probes from the first and second probe sets; (2) assigning a nucleotide in the target sequence as the complement of the interrogation position of the probe having the greater specific hybridization; and (3)

sequence has been assigned.

25. The method of claim 23, wherein the array further comprises third and fourth probe sets, each comprising a corresponding probe for each probe in the first probe set, the probes in the second, third and fourth probe sets being identical to a sequence comprising the corresponding probe from the first probe set or a subsequence of at least six nucleotides thereof that includes the at least one interrogation position, except that the at least one interrogation position is occupied by a different nucleotide in each of the four corresponding probes from the four probe sets; and the comparing step comprises comparing the relative specific hybridization of four corresponding probes from the first, second, third and fourth probe groups.

26. The method of claim 25, wherein: the reference sequence includes a site of a mutation in the biotransformation gene and a silent polymorphism in or flanking the biotransformation gene; the target nucleic acid comprises one or more different alleles of the biotransformation gene; and the relative specific hybridization of probes having an interrogation position aligned with the silent polymorphism indicates the number of different alleles and the relative specific hybridization of probes having an interrogation position aligned with the mutation indicates whether the mutation is present in at least one of the alleles.

27. The method of claim 25, wherein the determining comprises: (1) comparing the relative specific hybridization of four corresponding probes from the first, second, third and fourth probe sets; (2) assigning a nucleotide in the target sequence as the complement of the interrogation position of the probe having the greatest specific hybridization; (3) repeating (1) and (2) until each nucleotide of interest in the target sequence has been assigned.

28. A method of comparing a target nucleic acid with a reference sequence comprising a predetermined sequence of nucleotides, the method comprising: (a) hybridizing the target nucleic acid to an array of **oligonucleotide** probes immobilized on a solid support, the array comprising: a perfectly matched probe comprising a segment of at least six nucleotides exactly complementary to a subsequence of a reference sequence, the segment having a plurality of interrogation positions respectively corresponding to a plurality of nucleotides in the reference sequence, wherein the reference sequence is from a biotransformation gene; for each interrogation position, three mismatched probes, each identical to a sequence comprising the perfectly matched probe or a subsequence of at least six nucleotides thereof including the plurality of interrogation positions, except in the interrogation position, which is occupied by a different nucleotide in each of the three mismatched probes and the perfectly matched probe; (b) for each interrogation position, (1) comparing the relative specific hybridization of the three mismatched probes and the perfectly matched probe; (2) assigning a nucleotide in the target sequence as the complement of the interrogation position of the probe having the greatest specific hybridization.

29. The method of claim 28, wherein the target sequence has an undetermined substitution relative to the reference sequence, and the method assigns a nucleotide to the substitution.

30. A method of screening a patient for capacity to metabolize a drug, the method comprising: (a) hybridizing a tissue sample from the patient containing a target nucleic acid to an array of **oligonucleotide** probes immobilized on a solid support, the array comprising: (1) a first probe set comprising a plurality of probes, each probe comprising a segment of at least three nucleotides exactly complementary to a subsequence of the reference sequence from a biotransformation gene which metabolizes the drug, the segment including at least one interrogation position complementary to a corresponding nucleotide in the reference sequence, (2) a second probe set comprising a corresponding probe for each probe in the first probe set, the corresponding probe in the second probe set being identical to a sequence comprising the corresponding probe from the first probe set or a subsequence of at least three nucleotides thereof that includes the at least one interrogation position, except that the at least one interrogation position is occupied by a different nucleotide in each of the two corresponding probes from the first and second probe sets; wherein, the probes in the first probe set have at least three interrogation positions respectively corresponding to each of at least three nucleotides in the reference sequence, and (b) determining which probes, relative to one another, in the first and second probe sets specifically hybridize to the target nucleic acid, the relative specific hybridization of corresponding probes in the first and second probe sets indicating whether the target sequence contains a mutation relative to the reference sequence, which, if present, impairs

31. A method of conducting a clinical trial on a drug, the method comprising: (a) obtaining a tissue sample containing a target nucleic acid from each of a pool of patients; (b) for each tissue sample, hybridizing the target nucleic acid to an array of **oligonucleotide** probes immobilized on a solid support, the array comprising: (1) a first probe set comprising a plurality of probes, each probe comprising a segment of at least three nucleotides exactly complementary to a subsequence of the reference sequence from a biotransformation gene, the segment including at least one interrogation position complementary to a corresponding nucleotide in the reference sequence, (2) a second probe set comprising a corresponding probe for each probe in the first probe set, the corresponding probe in the second probe set being identical to a sequence comprising the corresponding probe from the first probe set or a subsequence of at least three nucleotides thereof that includes the at least one interrogation position, except that the at least one interrogation position is occupied by a different nucleotide in each of the two corresponding probes from the first and second probe sets; wherein, the probes in the first probe set have at least three interrogation positions respectively corresponding to each of at least three nucleotides in the reference sequence; (c) determining which probes, relative to one another, in the first and second probe sets specifically hybridize to the target nucleic acid, the relative specific hybridization of corresponding probes in the first and second probe sets indicating whether the target sequence contains a mutation relative to the reference sequence selecting a subpool of patients having a target sequence free of the mutation; and (d) administering the drug to the subpool of patients to determine efficacy.

32. The method of claim 31, further comprising combining the drug with a pharmaceutical carrier to form a pharmaceutical composition.

L5 ANSWER 87 OF 112 USPATFULL on STN

2001:165578 Method for producing nucleic acid strand immobilized carrier.

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US 2001024788 A1 20010927

APPLICATION: US 2001-791704 A1 20010226 (9)

PRIORITY: JP 2000-74490 20000316

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Using carrier having a template nucleic acid strand immobilized thereon, a probe nucleic acid strand is synthesized along the template strand, and the synthesized probe strand is immobilized on a separate substrate by utilizing an electric field to thereby manufacture a nucleic acid strand immobilized array simply and at low costs. By constructing the substrate of the array formed of an electrode, a DNA array enabling electric detection of DNAs can be obtained.

CLM What is claimed is:

1. A method for producing a nucleic acid immobilized carrier which has a second substrate and a second nucleic acid strand of a predetermined sequence immobilized on the second substrate, the method comprising the steps of: preparing a first nucleic acid immobilized carrier which has a first nucleic acid strand immobilized on a first substrate, the first nucleic acid strand having a nucleotide sequence complementary to that of the second nucleic acid strand; synthesizing a second nucleic acid strand along the first nucleic acid strand in a nucleic acid synthesizing solution, the second nucleic acid strand having a nucleotide sequence complementary to that of the first nucleic acid; disposing a second substrate such that the second substrate faces one side of the first substrate, on which sides the first nucleic acid strand are immobilized; and applying an electric field toward the first substrate from the second substrate to cause migration of the second nucleic acid strand to the surface of the second substrate, and immobilization of the second nucleic acid strand on the surface of the second substrate.

2. The method according to claim 1, wherein the migration and the immobilization of said second nucleic acid strand is carried out in an electrolytic solution under the condition which denature a double-stranded nucleic acid into a single-stranded nucleic acid.

3. The method according to claim 1, wherein the step of applying an electric field toward said first substrate from said second substrate is performed by providing a pair of electrodes respectively disposed outside of each of said first substrate and said second substrate, and applying potential between the pair of electrodes.

4. The method according to claim 1, wherein a surface of said first substrate and/or said second substrate is made of an conductive material, and the step of applying an electric field directed toward

potential to the substrate.

5. The method according to claim 4, wherein the first and/or the second substrate is obtained by coating an insulator substrate with an conductive film and dividing the surface of the conductive film into a plurality of isolated electrode regions by insulation layer patterns formed on the conductive film, said isolated electrode regions being immobilized with the second nucleic acid strand or the first nucleic acid strand having a different sequences, respectively.

6. The method according to claim 3, wherein at least one of said second substrate and said first substrate are made of a polymer or a glass.

7. The method according to claim 1, wherein said first or second nucleic acid strand is selected from the group consisting of RNAs, DNAs, PNAs and analogues thereof.

8. The method according to any one of claim 1, wherein the bonding between said second nucleic acid strand and said second substrate is a bond selected from the group consisting of a covalent bond, affinity bond and electrostatic bond.

9. The method according to claim 4, wherein the surface of said second substrate is made of gold, and the bonding between said second nucleic acid strand and said second substrate is attained through the affinity bond between sulfur bonded to said second nucleic acid strand and said gold surface.

10. The method according to claim 1, wherein said nucleic acid synthesis solution comprises a **primer**, a nucleic acid synthase enzyme, a nucleotide monomer and an electrolyte.

11. The method according to claim 2, wherein said condition which denature a double-stranded nucleic acid into a single-stranded nucleic acid is the temperature above 90° C. of the electrolyte solution.

12. The method according to claim 11, wherein said nucleic acid immobilized carrier is used for gene detection, and the second nucleic acid strand is a probe.

L5 ANSWER 88 OF 112 USPATFULL on STN

2001:162993 Self initiating single primer amplification of nucleic acids.

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US 6294323 B1 20010925

APPLICATION: US 1993-46682 19930414 (8)

DOCUMENT TYPE: Utility; GRANTED.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method is disclosed for producing at least one copy of a pair of complementary single stranded polynucleotides. The method comprises forming, in the presence of nucleoside triphosphates and template dependent polynucleotide polymerase along each of the complementary single stranded polynucleotides, an extension of a polynucleotide primer. The polynucleotide primer is comprised of at least a sequence of 16 nucleotides terminating at its 3' end in a 2 to 9 nucleotide sequence (S1), which is complementary with the 3' ends of both of the complementary single stranded polynucleotides. The polynucleotide primer has at least an 8 nucleotide sequence (S2) that is 5' of S1, where S2 is 50 to 80% complementary to the nucleotide sequences contiguous with the 3' ends of the complementary single stranded polynucleotides. The extended polynucleotide primer and the single stranded polynucleotides are then dissociated.

CLM What is claimed is:

1. A method of producing at least one copy of a pair of complementary single stranded polynucleotide sequences, said method comprising: (a) forming in the presence of nucleoside triphosphates and template dependent polynucleotide polymerase along each of said complementary single stranded polynucleotide sequences, an extension of a single polynucleotide **primer**, said polynucleotide **primer** being comprised of at least a sequence of 16 nucleotides terminating at its 3' end in a 4 to 8 nucleotide sequence (S1) that is complementary with the 3' ends of both of said complementary single stranded polynucleotide sequences, wherein said polynucleotide **primer** has a specifically designed sequence of at least 8 nucleotides (S2) that is 5' of said S1, said S2 being 50 to 80% complementary to each of the nucleotide sequences contiguous with the 3' ends of said complementary single stranded polynucleotide sequences, and (b) dissociating said extended polynucleotide **primer** and said single stranded polynucleotides thereby producing said copy of said single stranded polynucleotide sequences.

2. The method of claim 1 wherein said S2 is at least 60% complementary with each of said nucleotide sequences contiguous with the 3' ends of said complementary single stranded polynucleotide sequences.
3. The method of claim 1 wherein said pair of single stranded polynucleotide sequences and said copy are DNA.
4. The method of claim 1 wherein said polynucleotide **primer** is 24 to 90 nucleotides in length.
5. The method of claim 1 wherein said S2 contains at least one ambiguous nucleotide capable of binding to A, T, G and C.
6. The method of claim 5 wherein said ambiguous nucleotide is inosine.
7. The method of claim 1 wherein said S1 is at least 75% G and C.
8. The method of claim 1 wherein said S2 comprises a series of multiplets of nucleotides, said multiplets taken in order corresponding to at least 50% complementary multiplets at respectively ordered positions located alternately within sequences contiguous with the 3' ends of each of said complementary single stranded polynucleotide sequences.
9. The method of claim 8 wherein said S2 consists solely of said multiplets.
10. The method of claim 8 wherein said multiplets are quartets.
11. The method of claim 1 wherein said template-dependent polynucleotide polymerase is a DNA polymerase and said nucleoside triphosphates are dATP, dGTP, dCTP, and dTTP.
12. The method of claim 1 wherein said method is carried out at at least a 100-fold excess concentration of said polynucleotide **primer** relative to the concentration of said single stranded polynucleotides.
13. A method comprising repetition of the steps of claim 1 wherein said copy of said pair of complementary single stranded polynucleotide sequences has bonded to each of its 3' ends a sequence that is identical to said sequence S2 and a sequence at each of the 5' ends that is complementary to said sequence S2.
14. A method comprising repetition of the steps of claim 1 wherein the number of copies of said pair of single stranded polynucleotide sequences is increased by at least a factor of a thousand.
15. A method of producing multiple copies of a polynucleotide sequence and its complement, which comprises: (a) providing in combination (1) a pair of complementary single stranded polynucleotides having said polynucleotide sequence and its complement, (2) a single polynucleotide **primer** being comprised of a sequence of 24 to 90 nucleotides terminating at its 3' end in a 2 to 9 nucleotide sequence (S1) that is complementary with the 3' ends of both of said polynucleotide sequences and its complement, wherein said polynucleotide **primer** has a specifically designed sequence of at least 8 nucleotides (S2) that is 5' of said S1, said S2 being 50 to 80% complementary to each of the nucleotide sequences contiguous with the 3' ends of said polynucleotide sequence and its complement, (3) nucleoside triphosphates, (4) template dependent polynucleotide polymerase and (b) incubating said combination under conditions for either wholly or partially sequentially or concomitantly (1) dissociating said polynucleotide sequence and its complement, (2) hybridizing said polynucleotide **primer** with the sequences at the 3' end of said polynucleotide sequence and its complement, (3) extending said polynucleotide **primer** along said polynucleotide sequence and its complement to provide a first complementary pair of extended polynucleotide **primers**, (4) dissociating said first complementary pair of extended polynucleotide **primers** from said polynucleotide sequence and its complement, (5) hybridizing single stranded extended polynucleotide **primers** from said first complementary pair with said polynucleotide **primer**, (6) extending said polynucleotide **primer** along said single stranded extended polynucleotide **primers** to provide a second complementary pair of extended polynucleotide **primers**, (7) dissociating said second complementary pair of extended polynucleotide **primers** from said first complementary pair of extended polynucleotide **primers**, and (8) repeating steps (5)-(7) above thereby producing multiple copies of said polynucleotide sequences.
16. The method of claim 15 wherein said S1 is 4 to 8 nucleotides in length.

with each of said nucleotide sequences contiguous with the 3' ends of said polynucleotide sequence and its complement.

18. The method of claim 15 wherein said polynucleotide sequence or its complement or said first or second extended polynucleotide **primer** is DNA.

19. The method of claim 15 wherein said S2 contains at least one ambiguous nucleotide capable of binding to A, T, G and C.

20. The method of claim 19 wherein said ambiguous nucleotide is inosine.

21. The method of claim 15 wherein said S2 contains 1 to 4 ambiguous nucleotides capable of binding to A, T, G and C.

22. The method of claim 15 wherein said S1 is at least 75% G and C.

23. The method of claim 15 wherein said S2 comprises a series of multiplets of nucleotides, said multiplets taken in order corresponding to at least 50% complementary multiplets at respectively ordered positions located alternately within sequences contiguous with the 3' ends of each of said complementary single stranded polynucleotides.

24. The method of claim 23 wherein said S2 consists solely of said multiplets.

25. The method of claim 23 wherein said multiplets are quartets.

26. The method of claim 15 wherein said template-dependent polynucleotide polymerase is a DNA polymerase and said nucleoside triphosphates are dATP, dGTP, dCTP, and dTTP.

27. The method of claim 15 wherein said method is carried out at at least a 100-fold excess concentration of said polynucleotide **primer** relative to the concentration of said polynucleotide sequence and its complement.

28. A method comprising repetition of the steps of claim 15 wherein extended polynucleotide **primer** has an inverted repeat wherein one of the sequences of the inverted repeat is identical to the polynucleotide **primer**.

29. A method comprising repetition of the steps (5)-(7) of claim 15 wherein the number of said copies of said polynucleotide sequence and its complement is increased by at least a factor of a thousand.

30. A method for determining the presence of a polynucleotide analyte in a sample suspected of containing said analyte, said analyte having complementary single stranded polynucleotide sequences and nucleotide sequences contiguous with the 3' ends of said complementary single stranded polynucleotide sequences, said method comprising the steps of: (a) providing in combination (1) said sample, (2) a polynucleotide **primer** comprised of at least a sequence of 16 nucleotides terminating at its 3' end in a 4 to 8 nucleotide sequence (S1) that is complementary with the 3' ends of both of said complementary single stranded polynucleotide sequences, wherein said polynucleotide **primer** has a specifically designed sequence of at least 8 nucleotides (S2) that is 5' of said S1, said S2 being 50 to 80% complementary to each of the nucleotide sequences contiguous with the 3' ends of said complementary single stranded polynucleotide sequences, (3) nucleoside triphosphates and (4) template dependent polynucleotide polymerase; (b) incubating said combination under conditions for either wholly or partially sequentially or concomitantly (1) dissociating complementary sequences of said analyte into single stranded polynucleotides, (2) hybridizing said polynucleotide **primer** with the 3' end of said single stranded polynucleotide sequences, (3) extending said polynucleotide **primer** along said single stranded polynucleotide sequences to provide a first complementary pair of extended polynucleotide **primers**, (4) dissociating said first complementary pair of extended polynucleotide **primers** from said single stranded polynucleotide sequences, (5) hybridizing single stranded extended polynucleotide **primers** from said first complementary pair with said polynucleotide **primer**, (6) extending said polynucleotide **primer** along said single stranded extended polynucleotide **primers** to provide a second complementary pair of extended polynucleotide **primers**, (7) dissociating said second complementary pair of extended polynucleotide **primers** from said single stranded extended polynucleotide **primers**, and (8) repeating steps (5)-(7) above, wherein steps (a) and (b) are performed wholly or partially sequentially or concomitantly; and (c) detecting extended polynucleotide **primers**, the presence thereof indicating the presence of said polynucleotide analyte.

with each of said nucleotide sequences contiguous with the 3' ends of said complementary single stranded polynucleotide sequences.

32. The method of claim 30 wherein said single stranded polynucleotide sequences or said first or second extended polynucleotide **primer** is DNA.

33. The method of claim 30 wherein said polynucleotide **primer** is 24 to 90 nucleotides in length.

34. The method of claim 30 wherein said S2 contains at least one ambiguous nucleotide capable of binding to A, T, G and C.

35. The method of claim 34 wherein said ambiguous nucleotide is inosine.

36. The method of claim 30 wherein said S1 is at least 75% G and C.

37. The method of claim 30 wherein said S2 comprises a series of multiplets of nucleotides, said multiplets taken in order corresponding to at least 50% complementary multiplets at respectively ordered positions located alternately at a sequence contiguous with the 3' ends of each of said complementary single stranded polynucleotide sequences.

38. The method of claim 37 wherein said S2 consists solely of said multiplets.

39. The method of claim 37 wherein said multiplets are quartets.

40. The method of claim 30 wherein said template-dependent polynucleotide polymerase is a DNA polymerase and said nucleoside triphosphates are dATP, dGTP, dCTP, and dTTP.

41. The method of claim 30 wherein said method is carried out at at least a 100-fold excess concentration of said polynucleotide **primer** relative to the concentration of said polynucleotide analyte.

42. A method comprising repetition of steps (5)-(7) of claim 30 wherein the number of said copies of said single stranded polynucleotide sequences is increased by at least a factor of a thousand.

43. The method of claim 30 wherein said polynucleotide **primer** is labeled with a reporter group.

44. The method of claim 43 wherein said reporter group is selected from the group consisting of ligands, fluorescers, chemiluminescers, catalysts, co-enzymes, radioactive substances, amplifiable polynucleotide sequences, surfaces and small organic molecules.

45. The method of claim 30 wherein said single stranded polynucleotides contain a sequence that when hybridized to its complementary sequence can be bound specifically by a receptor.

46. The method of claim 45 wherein said receptor is selected from the group consisting of repressors, activators, and restriction enzymes.

47. The method of claim 30 wherein said detection includes detection of a specific DNA sequence.

48. The method of claim 30 comprising identifying complementary single stranded polynucleotide sequences of said analyte, together with nucleotide sequences contiguous with the 3' ends thereof, and preparing said polynucleotide **primer**.

L5 ANSWER 89 OF 112 USPATFULL on STN

2000:164260 Arrays of modified nucleic acid probes and methods of use.

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US 6156501 20001205

APPLICATION: US 1996-630427 19960403 (8)

DOCUMENT TYPE: Utility; Granted.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Oligonucleotide analogue arrays attached to solid substrates and methods related to the use thereof are provided. The oligonucleotide analogues hybridize to nucleic acids with either higher or lower specificity than corresponding unmodified oligonucleotides. Target nucleic acids which comprise nucleotide analogues are bound to oligonucleotide and oligonucleotide analogue arrays.

CLM What is claimed is:

targets and **oligonucleotide** probes comprising an array of a plurality of **oligonucleotide** analogue probes having different sequences, wherein said **oligonucleotide** analogue probes are coupled to a solid substrate at known locations and wherein said plurality of **oligonucleotide** analogue probes are selected to bind to complementary **oligonucleotide** targets with a similar hybridization stability across the array.

2. The composition of claim 1, wherein at least one of said **oligonucleotide** analogue probes is selected to maintain hybridization specificity or mismatch discrimination with said complementary **oligonucleotide** targets.

3. The composition of claim 1, wherein at least one of said **oligonucleotide** analogue probes has increased the thermal stability between said **oligonucleotide** analogue probe and said complementary **oligonucleotide** target as compared to an **oligonucleotide** probe that is the perfect complement to the complementary **oligonucleotide** target with which said **oligonucleotide** analogue probe anneals.

4. The composition of claim 1, wherein at least one of said **oligonucleotide** analogue probes has decreased the thermal stability between said **oligonucleotide** analogue probe and said complementary **oligonucleotide** target as compared to an **oligonucleotide** probe that is the perfect complement to the complementary **oligonucleotide** target with which said **oligonucleotide** analogue probe anneals.

5. The composition of claim 2, wherein at least one of said **oligonucleotide** analogue probes has increased the thermal stability between said **oligonucleotide** analogue probe and said complementary **oligonucleotide** target as compared to an **oligonucleotide** probe that is the perfect complement to the complementary **oligonucleotide** target with which said **oligonucleotide** analogue probe anneals.

6. The composition of claim 2, wherein at least one of said **oligonucleotide** analogue probes has decreased the thermal stability between said **oligonucleotide** analogue probe and said complementary **oligonucleotide** target as compared to an **oligonucleotide** probe that is the perfect complement to the complementary **oligonucleotide** target with which said **oligonucleotide** analogue probe anneals.

7. The composition of claims 1-5 or 6, wherein said solid substrate is selected from the group consisting of silica, polymeric materials, glass, beads, chips, and slides.

8. The composition of claims 1-5 or 6, wherein said composition comprises an array of **oligonucleotide** analogue probes 5 to 20 nucleotides in length.

9. The composition of claims 1-5 or 6, wherein said array of **oligonucleotide** analogue probes comprises a nucleoside analogue with the formula ##STR3## wherein: the nucleoside analogue is not a naturally occurring DNA or RNA nucleoside; R¹ is selected from the group consisting of hydrogen, methyl, hydroxyl, alkoxy, alkythio, halogen, cyano, and azido; R² is selected from the group consisting of hydrogen, methyl, hydroxyl, alkoxy, alkythio, halogen, cyano, and azido; Y is a heterocyclic moiety; and wherein said nucleoside analogue is incorporated into the **oligonucleotide** analogue by attachment to a 3' hydroxyl of the nucleoside analogue, to a 5' hydroxyl of the nucleoside analogue, or both the 3' nucleoside and the 5' hydroxyl of the nucleoside analogue.

10. The composition of claims 1-5 or 6, wherein said array of **oligonucleotide** analogue probes comprises a nucleoside analogue with the formula ##STR4## wherein: the nucleoside analogue is not a naturally occurring DNA or RNA nucleoside; R¹ is selected from the group consisting of hydrogen, hydroxyl, methyl, methoxy, ethoxy, propoxy, allyloxy, propargyloxy, Fluorine, Chlorine, and Bromine; R² is selected from the group consisting of hydrogen, hydroxyl, methyl, methoxy, ethoxy, propoxy, allyloxy, propargyloxy, Fluorine, Chlorine, and Bromine; and Y is a base selected from the group consisting of purines, purine analogues pyrimidines, pyrimidine analogues, 3-nitropyrrole and 5-nitroindole; and wherein said nucleoside analogue is incorporated into the **oligonucleotide** analogue by attachment to a 3' hydroxyl of the nucleoside analogue, to a 5' hydroxyl of the nucleoside analogue, or both the 3' nucleoside and the 5' hydroxyl of the nucleoside analogue.

11. The composition of claims 1-5 or 6, wherein each probe of said plurality of **oligonucleotide** analogue probes has at least one **oligonucleotide** analogue, and wherein at least one of said **oligonucleotide** analogues comprises a peptide nucleic acid.

plurality of **oligonucleotide** analogue probes said array of **oligonucleotide** analogue probes is resistant to RNAase A.

13. The composition of claims 1-5 or 6, wherein said solid substrate is attached to over 1000 different **oligonucleotide** analogue probes.

14. The composition of claims 1-5 or 6, wherein each probe of said plurality of **oligonucleotide** analogue probes has at least one **oligonucleotide** analogue, and wherein at least one of said **oligonucleotide** analogues comprises 2'-O-methyl nucleotides.

15. The composition of claims 1-5 or 6, wherein said array of **oligonucleotide** analogue probes and said solid substrate comprises a plurality of different **oligonucleotide** analogue probes, each **oligonucleotide** analogue probes having the formula: Y--L¹--X¹--L²--X² wherein, Y is a solid substrate; X¹ and X² are complementary oligonucleotides containing at least one nucleotide analogue; L¹ is a spacer; L² is a linking group having sufficient length such that X¹ and X² form a double-stranded **oligonucleotide**.

16. The composition of claim 15, wherein said composition comprises a library of unimolecular double-stranded **oligonucleotide** analogue probes.

17. The composition of claims 1-5 or 6, wherein said array of **oligonucleotide** analogue probes comprises a conformationally restricted array of **oligonucleotide** analogue probes with the formula: --X¹¹--Z--X¹² wherein X¹¹ and X¹² are complementary **oligonucleotides** or **oligonucleotide** analogues and Z is a presented moiety.

18. The composition of claims 1-5 or 6, wherein each probe of said plurality of **oligonucleotide** analogue probes has at least one **oligonucleotide** analogue, and wherein at least one of said **oligonucleotide** analogues comprises a nucleotide with a base selected from the group of bases consisting of 5-propynyluracil, 5-propynylcytosine, 2-aminoadenine, 7-deazaguanine, 2-aminopurine, 8-aza-7-deazaguanine, 1H-purine, and hypoxanthine.

19. The composition of claims 1-5 or 6, wherein said plurality of **oligonucleotide** analogue probes are coupled to said solid substrate by light-directed chemical coupling.

20. The composition of claim 19, wherein said solid substrate is derivitized with a silane reagent prior to synthesis of said plurality of **oligonucleotide** analogue probes.

21. The composition of claims 1-5 or 6, wherein said plurality of **oligonucleotide** analogue probes are coupled to said solid substrate by flowing **oligonucleotide** analogue reagents over known locations of the solid substrate.

22. The composition of claim 21, wherein said solid substrate is derivitized with a silane reagent prior to synthesis of said plurality of **oligonucleotide** analogue probes.

23. The composition of claims 1-5 or 6, wherein at least one of plurality of said **oligonucleotide** analogue probes forms a first duplex with a target **oligonucleotide** sequence, wherein said **oligonucleotide** analogue probe has a corresponding **oligonucleotide** sequence that forms a second duplex with said target **oligonucleotide** sequence, wherein said second duplex is rich in A-T or G-C nucleotide pairs, and wherein said **oligonucleotide** analogue probe has at least one nucleotide analogue in place of an A, T, G, or C nucleotide of said corresponding **oligonucleotide** sequence at a position within said **oligonucleotide** analogue probe such that said first duplex has an increased hybridization stability than said second duplex.

24. The composition of claim 23, wherein said **oligonucleotide** analogue probe contains fewer bases than said corresponding **oligonucleotide** sequence.

25. The composition of claims 1-5 or 6, wherein said **oligonucleotide** analogue probe forms a first duplex with a target **oligonucleotide** sequence, wherein said **oligonucleotide** analogue probe has a corresponding **oligonucleotide** sequence that forms a second duplex with said target polynucleotide sequence, and wherein said **oligonucleotide** analogue probe is shorter than said corresponding polynucleotide sequence.

26. A composition for analyzing the interaction between an **oligonucleotide** target and an **oligonucleotide** probe comprising an

sequences hybridized to complementary **oligonucleotide** analogue targets, wherein said **oligonucleotide** analogue targets bind to complementary **oligonucleotide** probes with a similar hybridization stability across the array.

27. The composition of claim 26, wherein at least one of said **oligonucleotide** analogue target is selected to maintain hybridization specificity or mismatch discrimination with said complementary **oligonucleotide** probes.

28. The composition of claim 26, wherein at least one of said **oligonucleotide** analogue targets has increased the thermal stability between said **oligonucleotide** analogue target and said complementary **oligonucleotide** probe as compared to an **oligonucleotide** target that is the perfect complement to the complementary **oligonucleotide** probe with which said **oligonucleotide** analogue target anneals.

29. The composition of claim 26, wherein at least one of said **oligonucleotide** analogue targets has decreased the thermal stability between said **oligonucleotide** analogue target and said complementary **oligonucleotide** probe as compared to an **oligonucleotide** target that is the perfect complement to the complementary **oligonucleotide** probe with which said **oligonucleotide** analogue target anneals.

30. The composition of claim 27, wherein at least one of said **oligonucleotide** analogue targets has increased the thermal stability between said **oligonucleotide** analogue target and said complementary **oligonucleotide** probe as compared to an **oligonucleotide** target that is the perfect complement to the complementary **oligonucleotide** probe with which said **oligonucleotide** analogue target anneals.

31. The composition of claim 27, wherein at least one of said **oligonucleotide** analogue targets has decreased the thermal stability between said **oligonucleotide** analogue target and said complementary **oligonucleotide** probe as compared to an **oligonucleotide** target that is the perfect complement to the complementary **oligonucleotide** probe with which said **oligonucleotide** analogue target anneals.

32. The composition of claims 26-30 or 31, wherein the **oligonucleotide** analogue target is a **PCR** amplicon.

33. The composition of claims 26-30 or 31, wherein at least one of said plurality of **oligonucleotide** probes comprise at least one **oligonucleotide** analogue.

34. The composition of claims 26-30 or 31, wherein at least one target **oligonucleotide** analogue acid is an RNA nucleic acid.

35. A method analyzing interactions between an **oligonucleotide** target and an **oligonucleotide** probe, comprising the steps of: (a). synthesizing an **oligonucleotide** analogue array comprising a plurality of **oligonucleotide** analogue probes having different sequences, wherein said **oligonucleotide** analogue probes are coupled to a solid substrate at known locations, said solid substrate having a surface; (b). exposing said **oligonucleotide** analogue probe array to a plurality of **oligonucleotide** targets under hybridization conditions such that said plurality of **oligonucleotide** analogue probes bind to complementary **oligonucleotide** targets with a similar hybridization stability across the array; and (c). determining whether an **oligonucleotide** analogue probe of said **oligonucleotide** analogue probe array binds to at least one of said target nucleic acids.

36. The method in accordance of claim 35, wherein at least one of said **oligonucleotide** analogue probes is selected to maintain hybridization specificity or mismatch discrimination with said complementary **oligonucleotide** targets.

37. The method in accordance of claim 35, wherein at least one of said **oligonucleotide** analogue probes has increased the thermal stability between said **oligonucleotide** analogue probe and said complementary **oligonucleotide** target as compared to an **oligonucleotide** probe that is the perfect complement to the complementary **oligonucleotide** target with which said **oligonucleotide** analogue probe anneals.

38. The method in accordance of claim 35, wherein at least one of said **oligonucleotide** analogue probes has decreased the thermal stability between said **oligonucleotide** analogue probe and said complementary **oligonucleotide** target as compared to an **oligonucleotide** probe that is the perfect complement to the complementary **oligonucleotide** target with which said **oligonucleotide** analogue probe anneals.

39. The method in accordance of claim 36, wherein at least one of said

between said **oligonucleotide** analogue probe and said complementary **oligonucleotide** target as compared to an **oligonucleotide** probe that is the perfect complement to the complementary **oligonucleotide** target with which said **oligonucleotide** analogue probe anneals.

40. The method in accordance of claim 36, wherein at least one of said **oligonucleotide** analogue probes has decreased the thermal stability between said **oligonucleotide** analogue probe and said complementary **oligonucleotide** target as compared to an **oligonucleotide** probe that is the perfect complement to the complementary **oligonucleotide** target with which said **oligonucleotide** analogue probe anneals.

41. The method of claims 35-39 or 40, wherein said **oligonucleotide** target is selected from the group comprising genomic DNA, cDNA, unspliced RNA, mRNA, and rRNA.

42. The method of claims 35-39 or 40, wherein said target nucleic acid is amplified prior to said hybridization step.

43. The method of claims 35-39 or 40, wherein said plurality of **oligonucleotide** analogue probes is synthesized on said solid support by light-directed synthesis.

44. The method of claims 35-39 or 40, wherein said plurality of said **oligonucleotide** analogue probes is synthesized on said solid support by causing **oligonucleotide** analogue synthetic reagents to flow over known locations of said solid support.

45. The method of claims 35-39 or 40, wherein said step (a). comprises the steps of: i). forming a plurality of channels adjacent to the surface of said substrate; ii). placing selected reagents in said channels to synthesize **oligonucleotide** analogue probes at known locations; and iii). repeating steps i). and ii). thereby forming an array of **oligonucleotide** analogue probes having different sequences at known locations on said substrate.

46. The method of claims 35-39 or 40, wherein said solid substrate is selected from the group consisting of beads, slides, and chips.

47. The method of claims 35-39 or 40, wherein said solid substrate is comprised of materials selected from the group consisting of silica, polymers and glass.

48. The method of claims 35-39 or 40, wherein the **oligonucleotide** analogue probes of said array are synthesized using photoremovable protecting groups.

49. The method of claims 35-39 or 40, further comprising selectively incorporating MeNPoc onto the 3' or 5' hydroxyl of at least one nucleoside analogue and selectively incorporating said nucleoside analogue into at least one of said **oligonucleotide** analogue probes.

50. The method of claims 35-39 or 40, wherein at least one of said **oligonucleotide** analogue probes is synthesized from phosphoramidite nucleoside reagents.

51. A method of detecting an **oligonucleotide** target, comprising enzymatically copying an **oligonucleotide** target using at least one nucleotide analogue, thereby producing multiple **oligonucleotide** analogue targets, selecting said **oligonucleotide** analogue targets such that said **oligonucleotide** analogue targets bind to the complementary **oligonucleotide** probes coupled to a solid surface at known locations of an array with a similar hybridization stability across the array, hybridizing the **oligonucleotide** analogue targets to complementary **oligonucleotide** probes, and detecting whether at least one of said oligonucleotide analogue targets binds to said complementary **oligonucleotide** acid probe.

52. The method of claim 51, wherein at least one of said **oligonucleotide** analogue targets is selected to maintain hybridization specificity or mismatch discrimination with said complementary **oligonucleotide** probes.

53. The method of claim 51, wherein at least one of said **oligonucleotide** analogue targets has increased the thermal stability between said **oligonucleotide** analogue target and said complementary **oligonucleotide** probe as compared to an **oligonucleotide** target that is the perfect complement to the complementary **oligonucleotide** probe with which said **oligonucleotide** analogue target anneals.

54. The method of claim 51, wherein at least one of said **oligonucleotide** analogue targets has decreased the thermal stability

oligonucleotide probe as compared to an **oligonucleotide** target that is the perfect complement to the complementary **oligonucleotide** probe with which said **oligonucleotide** analogue target anneals.

55. The method of claim 52, wherein at least one of said **oligonucleotide** analogue targets has increased the thermal stability between said **oligonucleotide** analogue target and said complementary **oligonucleotide** probe as compared to an **oligonucleotide** target that is the perfect complement to the complementary **oligonucleotide** probe with which said **oligonucleotide** analogue target anneals.

56. The method of claim 52, wherein at least one of said **oligonucleotide** analogue targets has decreased the thermal stability between said **oligonucleotide** analogue target and said complementary **oligonucleotide** probe as compared to an **oligonucleotide** target that is the perfect complement to the complementary **oligonucleotide** probe with which said **oligonucleotide** analogue target anneals.

57. The method of claims 51-55 or 56, wherein the **oligonucleotide** probe array comprises at least one **oligonucleotide** analogue probe which is complementary to at least one of said **oligonucleotide** analogue targets.

58. A method of making an array of **oligonucleotide** probes, comprising providing a plurality of **oligonucleotide** analogue probes having at least one **oligonucleotide** analogue, said **oligonucleotide** analogue probes having different sequences at known locations on an array, selecting the **oligonucleotide** analogue probes to hybridize with complementary **oligonucleotide** target sequences under hybridization conditions such that said **oligonucleotide** analogue probes bind to complementary **oligonucleotide** targets with a similar hybridization stability, across the array.

59. The method of claim 58, wherein at least one of said **oligonucleotide** analogue probes is selected to maintain hybridization specificity or mismatch discrimination with said complementary **oligonucleotide** targets.

60. The method of claim 58, wherein at least one of said **oligonucleotide** analogue probes has increased the thermal stability between said **oligonucleotide** analogue probe and said complementary **oligonucleotide** target as compared to an **oligonucleotide** probe that is the perfect complement to the complementary **oligonucleotide** target with which said **oligonucleotide** analogue probe anneals.

61. The method of claim 58, wherein at least one of said **oligonucleotide** analogue probes has decreased the thermal stability between said **oligonucleotide** analogue probe and said complementary **oligonucleotide** target as compared to an **oligonucleotide** probe that is the perfect complement to the complementary **oligonucleotide** target with which said **oligonucleotide** analogue probe anneals.

62. The method of claim 59, wherein at least one of said **oligonucleotide** analogue probes has increased the thermal stability between said **oligonucleotide** analogue probe and said complementary **oligonucleotide** target as compared to an **oligonucleotide** probe that is the perfect complement to the complementary **oligonucleotide** target with which said **oligonucleotide** analogue probe anneals.

63. The method of claim 59, wherein at least one of said **oligonucleotide** analogue probes has decreased the thermal stability between said **oligonucleotide** analogue probe and said complementary **oligonucleotide** target as compared to an **oligonucleotide** probe that is the perfect complement to the complementary **oligonucleotide** target with which said **oligonucleotide** analogue probe anneals.

64. The method in accordance with claims 58-62, or 63, further comprising incorporating at least one **oligonucleotide** analogue into at least one of the **oligonucleotide** analogue probes of the array to reduce or prevent the formation of secondary structure in the **oligonucleotide** of the array.

65. The method in accordance with claims 58-62, or 63, further comprising incorporating at least one **oligonucleotide** analogue into at least one of the **oligonucleotide** target to reduce or prevent the formation of secondary structure in the target polynucleotide sequence.

66. The method in accordance with claims 58-62, or 63, further comprising incorporating at least one **oligonucleotide** analogue into at least one of the **oligonucleotide** analogue probes of the array to create secondary structure in the **oligonucleotide** of the array.

comprising incorporating a base selected from the group consisting of 5-propynyluracil, 5-propynylcytosine, 2-aminoadenine, 7-deazaguanine, 2-aminopurine, 8-aza-7-deazaguanine, 1H-purine, and hypoxanthine into the **oligonucleotide** analogue probes of the array.

68. The method of claim 67 further comprising selecting said at least one **oligonucleotide** analogue such that the **oligonucleotide** analogue probe is a homopolymer.

69. The method in accordance with claims 58-62, or 63, further comprising selecting said at least one **oligonucleotide** analogue from the group consisting essentially of **oligonucleotide** analogues comprising 2'-O-methyl nucleotides and **oligonucleotides** comprising a base selected from the group of bases consisting of 5-propynyluracil, 5-propynylcytosine, 7-deazaguanine, 2-aminoadenine, 8-aza-7-deazaguanine, 1H-purine, and hypoxanthine.

70. The method in accordance with claims 58-62 or 63, further comprising selecting said at least one **oligonucleotide** analogue such that **oligonucleotide** analogue probes comprises at least one peptide nucleic acid.

71. The method in accordance with claims 58-62, or 63, further comprising selecting said at least one **oligonucleotide** analogue to increase image brightness when the **oligonucleotide** target and the **oligonucleotide** analogue probe hybridize in the presence of a fluorescent indicator, in comparison to a **oligonucleotide** probe without **oligonucleotide** analogs.

72. The method in accordance with claims 58-62, or 63, further comprising providing said plurality of **oligonucleotide** analogue probes in an array with at least 1000 other **oligonucleotide** analogue probes.

L5 ANSWER 90 OF 112 USPATFULL on STN

2000:128125 Nucleic acid amplification using single primer.

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US 6124090 20000926

APPLICATION: US 1995-438149 19950509 (8)

DOCUMENT TYPE: Utility; Granted.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method is disclosed for determining the presence of a polynucleotide analyte in a sample suspected of containing the analyte. The method comprises (a) forming as a result of the presence of an analyte a single stranded polynucleotide comprising a target polynucleotide binding sequence flanked by first and second polynucleotide sequences that differ from the sequence of the analyte or a sequence complementary to the analyte sequence, (b) forming multiple copies of the single stranded polynucleotide, and (c) detecting the single stranded polynucleotide. Also disclosed is a method of producing at least one copy of a single stranded polynucleotide. The method comprises (a) forming in the presence of nucleoside triphosphates and template dependent polynucleotide polymerase an extension of a polynucleotide primer at least the 3'-end of which has at least a 10 base sequence hybridizable with a second sequence flanking the 3'-end of the single stranded polynucleotide, the second sequence being partially or fully complementary with at least a 10 base first sequence flanking the 5' end of the single stranded polynucleotide, (b) dissociating the extended polynucleotide primer and the single stranded polynucleotide, (c) repeating step a and (d) dissociating the extended polynucleotide primer and the copy of the single stranded polynucleotide.

CLM What is claimed is:

1. A method for determining the presence of a polynucleotide analyte in a sample suspected of containing said analyte, which comprises the steps of: (a) forming as a result of the presence of said analyte a single stranded polynucleotide flanked by at least 50% complementary first and second flanking sequences wherein said second flanking sequence is 3' of said first flanking sequence, (b) forming multiple copies of said single stranded polynucleotide and said flanking sequences in the presence of nucleoside triphosphates, template dependent polynucleotide polymerase and a polynucleotide **primer** that has at least a 10 base sequence hybridizable with said second flanking sequence, and (c) detecting said single stranded polynucleotide, the presence thereof indicating the presence of said analyte.

2. The method of claim 1 wherein said first and second flanking

3. The method of claim 2 wherein said multiple copies are formed by incubating said assay medium under conditions for either wholly or partially sequentially or concomitantly (1) hybridizing a single stranded polynucleotide **primer** at its 3'-end to the flanking sequence at the 3'-end of said single stranded polynucleotide, (2) extending said polynucleotide **primer** in the presence of nucleoside triphosphates and a polynucleotide polymerase to provide a first extended polynucleotide **primer**, (3) dissociating said first extended polynucleotide **primer** and said single stranded polynucleotide, (4) hybridizing said first extended polynucleotide **primer** with said polynucleotide **primer**, (5) extending said polynucleotide **primer** along said first extended polynucleotide **primer** to provide a second extended polynucleotide **primer**, (6) dissociating said second extended polynucleotide **primer** from said first extended polynucleotide **primer**, and (7) repeating steps (4)-(6) above.

4. The method of claim 1 wherein said polynucleotide analyte is DNA.

5. The method of claim 3 wherein said polynucleotide **primer** is 10 to 100 nucleotides in length.

6. A method for determining the presence of a polynucleotide analyte in a sample suspected of containing said analyte, which comprises the steps of: (a) forming as a result of the presence of said analyte a single stranded polynucleotide flanked by at least 80% complementary first and second flanking sequences, each comprised of at least 15 bases, (b) forming in the presence of nucleoside triphosphates and template dependent polynucleotide polymerase an extension of a polynucleotide **primer** at least the 3'-end of which can hybridize with the flanking sequence at the 3'-end of said single stranded polynucleotide sequence, wherein steps (a) and (b) can be performed wholly or partially sequentially or concomitantly, and (c) detecting extended polynucleotide **primer** containing a sequence identical to and/or complementary with said polynucleotide sequence, the presence thereof indicating the presence of said analyte.

7. The method of claim 6 wherein said first and second flanking sequences are fully complementary.

8. The method of claim 6 wherein a portion of said polynucleotide **primer** is labeled with one reporter group and a portion is labeled with a second reporter group.

9. The method of claim 6 wherein said polynucleotide analyte is DNA.

10. The method of claim 6 wherein said polynucleotide **primer** is 20 to 100 nucleotides in length.

11. The method of claim 6 wherein said template-dependent polynucleotide polymerase is a DNA polymerase and said nucleoside triphosphates are dATP, dGTP, dCTP, and dTTP.

12. The method of claim 6 wherein said method is carried out at a substantially excess concentration of said polynucleotide **primer** relative to the concentration of said single stranded polynucleotide.

13. The method of claim 6 wherein step (b) is repeated such that the number of said copies of said extended polynucleotide **primer** formed is increased by at least a factor of three.

14. The method of claim 6 wherein said polynucleotide **primer** is labeled with a reporter group.

15. The method of claim 14 wherein said reporter group is selected from the group consisting of, fluorescers, chemiluminescers, catalysts, co-enzymes, radioactive substances, amplifiable polynucleotide sequences, and small organic molecules.

16. The method of claim 6 wherein said polynucleotide **primer** is labeled with a ligand.

17. The method of claim 6 wherein said single stranded polynucleotide contains a sequence that when hybridized to its complementary sequence can be bound specifically by a receptor.

18. The method of claim 17 wherein said receptor is selected from the group consisting of repressors, activators, and restriction enzymes.

19. The method of claim 6 wherein said single stranded polynucleotide contains a sequence at its end that when hybridized to its complementary sequence, can be bound specifically by a receptor, and said extended

extended polynucleotide **primer**.

20. A method for determining the presence of a polynucleotide analyte in a sample suspected of containing said analyte, which comprises; (a) contacting said sample with first and second polynucleotide probes, said first probe comprising (i) a target polynucleotide binding sequence at its 3'-end complementary to a first portion of one strand of said analyte and (ii) a first flanking sequence and said second probe comprising (i) a target polynucleotide binding sequence at its 5' end complementary to a second portion of the same strand of said analyte and (ii) a second flanking sequence wherein said first and second flanking sequences are at least 65% complementary, under conditions for binding of said first and second probes with said analyte, (b) providing conditions for ligating said first and second polynucleotide probes to one another only when bound to said analyte, (c) forming in the presence of nucleoside triphosphates and template dependent polynucleotide polymerase an extension of a polynucleotide **primer** at least the 3'-end of which can hybridize with said second flanking sequence, and (d) detecting extended polynucleotide **primer** containing a sequence complementary to said first probe, the presence thereof indicating the presence of said analyte.

21. The method of claim 20 wherein said polynucleotide analyte is DNA.

22. The method of claim 20 wherein said polynucleotide **primer** is 10 to 100 nucleotides in length.

23. The method of claim 20 wherein said template-dependent polynucleotide polymerase is a DNA polymerase and said nucleoside triphosphates are dATP, dGTP, dCTP, and DTTP.

24. The method of claim 20 wherein said method is carried out at a substantially excess concentration of said polynucleotide **primer** relative to the concentration of said polynucleotide analytes.

25. The method of claim 20 wherein step (c) is repeated such that the number of said copies of said extension polynucleotide **primer** formed is increased by at least a factor of three.

26. The method of claim 20 wherein said polynucleotide **primer** is labeled with a reporter group.

27. The method of claim 26 wherein said reporter group is selected from the group consisting of, fluorescers, chemiluminescers, catalysts, co-enzymes, radioactive substances, amplifiable polynucleotide sequences, and small organic molecules.

28. The method of claim 26 wherein said first probe contains an operator sequence between said target polynucleotide binding sequence complementary to said analyte and said first flanking complementary sequence.

29. The method of claim 20 wherein said detection includes detection of a specific DNA sequence.

30. The method of claim 26 wherein separate molecules of polynucleotide **primer** are labeled with a group capable of becoming bound to a surface.

31. The method of claim 20 wherein said first and second polynucleotide probes when hybridized with said analyte are less than two nucleotides apart and conditions for ligating said first and second polynucleotide probes include addition of a ligase.

32. The method of claim 20 wherein said first and second polynucleotide probes when hybridized with said analyte are at least one nucleotide apart and conditions for ligating said first and second polynucleotide probes include (a) adding nucleotide triphosphates and a polynucleotide polymerase to render the hybridized portions contiguous and (b) adding ligase to ligate said hybridized portions.

33. A method for detecting the presence of a polynucleotide analyte in a sample suspected of containing said analyte, which comprises: (a) hybridizing to said analyte a first polynucleotide probe and a second polynucleotide probe, said first polynucleotide probe having (i) a sequence S1 that is at least 10 nucleotides in length and at least 50% complementary to a sequence S2 of said second polynucleotide probe and (ii) a sequence S3 that is 3' of said S1 and having a free 3'-end, said second polynucleotide probe having a sequence S4 that is 5' of said S2, said S4 having a free 5'-end, wherein the 3'-end of said S3 and the 5'-end of said S4 are ligatable, said first polynucleotide probe hybridizing to said analyte at a portion thereof that is 3' of the portion to which said second polynucleotide probe hybridizes to said

second polynucleotide probes, (c) forming in the presence of nucleoside triphosphates and template dependent polynucleotide polymerase an extension of a polynucleotide **primer** at least the 3'-end of which hybridizes with said S2 of said ligated first and second polynucleotide probes, wherein steps (a) and (b) and (c) are performed wholly or partially sequentially or concomitantly and, (d) detecting extended polynucleotide **primer** and/or a sequence identical to and/or complementary with at least that portion of said ligated first and second polynucleotide probes, the presence thereof indicating the presence of said analyte.

34. The method of claim 33 wherein said first and second flanking sequences are capable of forming an inverted repeat.

35. The method of claim 33 wherein said polynucleotide analyte is DNA.

36. The method of claim 33 wherein said S1 and S2 are fully complementary.

37. The method of claim 33 wherein said S3 and S4 are fully complementary to separate portions of said analyte.

38. The method of claim 33 wherein said polynucleotide **primer** is 15 to 100 nucleotides in length.

39. The method of claim 33 wherein said template-dependent polynucleotide polymerase is a DNA polymerase and said nucleoside triphosphates are dATP, dGTP, dCTP, and dTTP.

40. The method of claim 33 wherein said method is carried out at a substantially excess concentration of said polynucleotide **primer** relative to the concentration of said polynucleotide analyte.

41. The method of claim 33 wherein step (b) is repeated such that the number of said copies of said extended polynucleotide **primer** formed is increased by at least a factor of three.

42. The method of claim 33 wherein said polynucleotide **primer** is labeled with a reporter group.

43. The method of claim 42 wherein said reporter group is selected from the group consisting of fluorescers, chemilumescers, catalysts, co-enzymes, radioactive substances, amplifiable polynucleotide sequences, and small organic molecules.

44. The method of claim 43 wherein said first polynucleotide probe contains an operator sequence.

45. The method of claim 33 wherein said detection includes detection of a specific DNA sequence.

46. The method of claim 33 wherein said S3 and S4 when hybridized with said analyte are less than two nucleotides apart and conditions for ligation of said S3 and S4 include addition of a ligase.

47. The method of claim 33 wherein said S3 and S4 when hybridized with said analyte are at least one nucleotide apart and ligation of said S3 and S4 includes: (a) adding nucleoside triphosphates and a polynucleotide polymerase to render said S3 and S4 contiguous, and (b) adding ligase to ligate said hybridized portions.

48. A method for determining the presence of a polynucleotide analyte in a sample suspected of containing said analyte, which comprises; (a) combining in an assay medium said sample with a first polynucleotide probe and a second polynucleotide probe, said first probe comprising a sequence S1 at least 10 nucleotides in length and at least 80% complementary with a sequence S2 in said second probe, said first probe being attached at its 3'-end to a sequence having a portion S3 that is hybridizable with a first portion of a single strand of said analyte wherein said S2 is attached at its 5'-end to a sequence having a portion S4 that is hybridizable with a second portion of said single strand of said analyte, (b) incubating said assay medium under conditions for binding of said first and second probes with said analyte, said first and second probes being ligatable, or capable of being rendered ligatable, to one another only when bound to said analyte, (c) ligating said first and second probes to form ligated first and second probes with the proviso that, if necessary, where they are not otherwise ligatable, said first and second probes are rendered ligatable, (d) combining said assay medium with a polynucleotide **primer** having a 3' terminal sequence complementary to said S2 in said second probe, (e) incubating said assay medium under conditions for either wholly or partially sequentially or concomitantly (1) dissociating any internally

polynucleotide **primer** with said ligated first and second probes, (3) extending said polynucleotide **primer** along said ligated first and second probes, (4) dissociating said hybridized extended polynucleotide **primer** and said ligated first and second probes, (5) hybridizing said extended polynucleotide **primer** with said polynucleotide **primer**, (6) extending said polynucleotide **primer** along said extended polynucleotide **primer**, (7) dissociating said hybridized extended **primer**, and (8) repeating steps (5)-(7) above, wherein steps (a)-(d) are carried out wholly or partially sequentially or concomitantly followed by step (e), and (f) detecting said extended **primer**, the presence thereof indicating the presence of said analyte.

49. The method of claim 48 wherein said polynucleotide analyte is DNA.

50. The method of claim 48 wherein said S1 is full complementary to said S2.

51. The method of claim 48 wherein said polynucleotide **primer** is 10 to 100 nucleotides in length.

52. The method of claim 48 wherein said template-dependent polynucleotide polymerase is a DNA polymerase and said nucleoside triphosphates are dATP, dGTP, dCTP, and dTTP.

53. The method of claim 48 wherein said method is carried out at a substantially excess concentration of said polynucleotide **primer** relative to the concentration of said polynucleotide analyte.

54. The method of claim 48 wherein the number of said copies of said extended polynucleotide **primer** formed is increased by at least a factor of three.

55. The method of claim 48 wherein said polynucleotide **primer** is labeled with a reporter group.

56. The method of claim 55 wherein said reporter group is selected from the group consisting of, fluorescers, chemilumescers, radioactive substances, co-enzymes, catalysts, amplifiable polynucleotide sequences, and small organic molecules.

57. The method of claim 55 wherein one of said probes contains an operator or repressor binding sequence.

58. The method of claim 55 wherein separate molecules of polynucleotide **primer** are labelled with a group capable of becoming bound to a surface.

59. The method of claim 48 wherein said detection includes detection of a specific DNA sequence.

60. The method of claim 48 wherein a portion of said polynucleotide **primer** is labeled with fluorescein and another portion of said polynucleotide **primer** is labeled with biotin and said detecting includes (a) adding a support to which avidin is bound, (b) separating said support from said assay medium, and (c) examining said support for the presence of fluorescein.

61. The method of claim 48 wherein said extending of said polynucleotide **primer** along said ligated first and second probes forms at least part of an operator and said detecting includes: (a) adding a repressor bound to a solid support and (b) detecting a sequence bound to said solid support that is identical or complementary to a sequence in said first polynucleotide probe.

62. The method of claim 61 wherein said sequence is detected by the binding of a labeled polynucleotide probe to said first polynucleotide probe sequence.

63. The method of claim 48 wherein said first and second polynucleotide probes when hybridized with said analyte are less than two nucleotides apart and conditions for ligating said first and second polynucleotide probes include addition of a ligase.

64. The method of claim 48 wherein said first and second polynucleotide probes when hybridized with said analyte are at least one nucleotide apart and conditions for ligating said first and second polynucleotide probes include (a) adding nucleoside triphosphates and a polynucleotide polymerase to render the hybridized portions contiguous and (b) adding ligase to ligate said hybridized portions.

65. A method for determining the presence of a polynucleotide analyte in a sample suspected of containing said analyte, which comprises: (a) contacting said sample with a first polynucleotide probe and a second

S1 that is at least 10 nucleotides in length and at least 80% complementary to a second sequence S2 in said second probe and (2) a sequence S3 complementary to a portion of said analyte other than a portion complementary to a sequence S4 of said second probe under conditions for binding of said first and second probes with said analyte, (b) ligating said first and second probes to one another to form ligated first and second probes, (c) forming in the presence of nucleoside triphosphates and template dependent polynucleotide polymerase an extension of a polynucleotide **primer** at least a portion of which is hybridized with said S1 or said S2 of said ligated first and second probes, wherein steps (a) and (b) and (c) are carried out wholly or partially sequentially or concomitantly and (d) detecting said extended polynucleotide **primer** and/or said ligated first and second probes, the presence thereof indicating the presence of said analyte.

L5 ANSWER 91 OF 112 USPATFULL on STN

2000:124779 Detection of nucleic acids by target-catalyzed product formation.

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US 6121001 20000919

APPLICATION: US 1999-440363 19991115 (9)

DOCUMENT TYPE: Utility; Granted.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method is disclosed for modifying an oligonucleotide, which method has application to the detection of a polynucleotide analyte. An oligonucleotide is reversibly hybridized with a polynucleotide, for example, a polynucleotide analyte, in the presence of a 5'-nuclease under isothermal conditions. The polynucleotide analyte serves as a recognition element to enable a 5'-nuclease to cleave the oligonucleotide to provide (i) a first fragment that is substantially non-hybridizable to the polynucleotide analyte and (ii) a second fragment that lies 3' of the first fragment (in the intact oligonucleotide) and is substantially hybridizable to the polynucleotide analyte. At least a 100-fold molar excess of the first fragment and/or the second fragment are obtained relative to the molar amount of the polynucleotide analyte. The presence of the first fragment and/or the second fragment is detected, the presence thereof indicating the presence of the polynucleotide analyte. The method has particular application to the detection of a polynucleotide analyte such as DNA. Kits for conducting methods in accordance with the present invention are also disclosed.

CLM What is claimed is:

1. A method for modifying an **oligonucleotide**, the method comprising incubating under isothermal conditions the **oligonucleotide**, a polynucleotide, and a nuclease, wherein the **oligonucleotide** and the polynucleotide form a complex comprising said **oligonucleotide** and said polynucleotide in which at least a portion of the **oligonucleotide** is hybridized to the polynucleotide, wherein the isothermal conditions are at or near the melting temperature of the complex and wherein the **oligonucleotide**, when the portion is hybridized to the polynucleotide, is cleaved by the nuclease to provide (i) a first fragment that is substantially non-hybridizable to the polynucleotide and includes no more than five nucleotides from the 5'-end of the portion and (ii) a second fragment that is 3' of the first fragment with reference to the intact **oligonucleotide** and is substantially hybridizable to the polynucleotide, thereby modifying said **oligonucleotide**, wherein said first fragment and said second fragment are continuously produced under said isothermal conditions.

2. The method of claim 1 wherein the amounts of fragments that are formed are at least 100-fold larger than the amount of the polynucleotide.

3. The method of claim 1 wherein a second **oligonucleotide** is present during the incubating, wherein the second **oligonucleotide** hybridizes to a site on the polynucleotide that is in the 3' direction from the site at which the **oligonucleotide** is hybridized and wherein the second **oligonucleotide** is substantially non-reversibly hybridized to the polynucleotide under the isothermal conditions.

4. The method of claim 3 wherein the second **oligonucleotide** hybridizes to the polynucleotide at a site contiguous with the site on the polynucleotide at which the **oligonucleotide** hybridizes.

5. The method of claim 4 wherein the amounts of fragments that are formed are at least 100-fold larger than the amount of the polynucleotide.

forming a mixture comprising a sample suspected of containing a polynucleotide analyte, an **oligonucleotide** and a nuclease, (b) incubating the mixture at a temperature at which the **oligonucleotide** reversibly hybridizes to the polynucleotide analyte, wherein the **oligonucleotide** has a 5' portion which does not substantially hybridizes with the polynucleotide analyte at said temperature and a 3' portion which substantially hybridizes with the polynucleotide analyte at said temperature, thereby forming a polynucleotide complex comprising at least the polynucleotide analyte and the **oligonucleotide**, wherein the complex serves as a substrate for the nuclease, and wherein during said incubating the nuclease cleaves the **oligonucleotide** when the **oligonucleotide** is hybridized to the polynucleotide analyte to continuously produce (i) a first fragment that is substantially non-hybridizable to the polynucleotide analyte and includes no more than five nucleotides from the 5'-end of the portion which substantially hybridizes to the polynucleotide analyte, and (ii) a second fragment that is 3' of the first fragment with reference to the intact **oligonucleotide** and is substantially hybridizable to the polynucleotide analyte, and (c) detecting the presence of the first fragment, the second fragment, or the first and second fragments, the presence thereof indicating the presence of the polynucleotide analyte.

7. The method of claim 6 wherein at least one of the first fragment and the second fragment has a label.

8. The method of claim 6 wherein the first fragment includes no more than one nucleotide from the 5'-end of the portion of the **oligonucleotide** that substantially hybridizes to the polynucleotide analyte.

9. The method of claim 6 wherein the mixture further comprises a second **oligonucleotide** that substantially fully hybridizes to a site on the polynucleotide analyte that is in the 3' direction from the site at which the **oligonucleotide** hybridizes and wherein the second **oligonucleotide** is substantially fully hybridized to the polynucleotide analyte at the temperature.

10. The method of claim 9 wherein the second **oligonucleotide** hybridizes to the polynucleotide analyte at a site contiguous with the site on the polynucleotide analyte at which the **oligonucleotide** hybridizes.

11. A method for detecting a polynucleotide analyte, the method comprising: (a) providing in combination a medium suspected of containing the polynucleotide analyte, a molar excess, relative to the suspected concentration of the polynucleotide analyte, of a first **oligonucleotide** at least a portion of which is reversibly hybridizes with the polynucleotide analyte under isothermal conditions, a 5'-nuclease, and a second **oligonucleotide** that hybridizes to a site on the polynucleotide analyte that is in the 3' direction of the site at which the first **oligonucleotide** reversibly hybridizes wherein the polynucleotide analyte is substantially fully hybridized to the second **oligonucleotide** under the isothermal conditions, (b) reversibly hybridizing under the isothermal conditions the polynucleotide analyte and the first **oligonucleotide**, wherein the first **oligonucleotide**, when hybridized to the polynucleotide analyte, is cleaved by the 5'-nuclease as a result of the presence of the polynucleotide analyte to provide, in at least a 100-fold molar excess of the polynucleotide analyte, (i) a first fragment that is substantially non-hybridizable to the polynucleotide analyte and (ii) a second fragment that is 3' of the first fragment with reference to the intact first **oligonucleotide** and is substantially hybridizable to the polynucleotide analyte, wherein said first fragment and said second fragment are continuously produced under said isothermal conditions, and (c) detecting the presence of the first fragment, the second fragment, or the first and second fragments, the presence thereof indicating the presence of the polynucleotide analyte.

12. The method of claim 11 wherein the first fragment and/or the second fragment has a label.

13. The method of claim 12 wherein the label is selected from the group consisting of a member of a specific binding pair, dyes, fluorescent molecules, chemiluminescers, coenzymes, enzyme substrates, radioactive groups and suspendible particles.

14. The method of claim 11 wherein the polynucleotide analyte is DNA.

15. The method of claim 11 wherein the first fragment includes no more than 5 nucleotides from the 5'-end of the portion of the first **oligonucleotide** that is reversibly hybridizes to the polynucleotide analyte.

16. The method of claim 11 wherein the second **oligonucleotide** hybridizes to the polynucleotide analyte at a site contiguous with the site on the polynucleotide analyte at which the first **oligonucleotide** reversibly hybridizes.

17. A method for detecting a DNA analyte, the method comprising: (a) providing in combination a medium suspected of containing the DNA analyte, a first **oligonucleotide** at least a portion of which reversibly hybridizes with the DNA analyte under isothermal conditions, a 5' nuclease, and a second **oligonucleotide** that hybridizes to a site on the DNA analyte that is in the 3' direction from the site at which the first **oligonucleotide** reversibly hybridizes wherein the DNA analyte is substantially fully hybridized to the second **oligonucleotide** under the isothermal conditions, (b) reversibly hybridizing the DNA analyte and the first **oligonucleotide** under the isothermal conditions, wherein the first **oligonucleotide**, when hybridized to the DNA analyte, is cleaved by the 5'-nuclease to provide (i) a first fragment that is substantially non-hybridizable to the DNA analyte and (ii) a second fragment that is 3' of the first fragment with reference to the intact first **oligonucleotide** and is substantially hybridizable to the DNA analyte, wherein at least a 100-fold molar excess, relative to the DNA analyte, of the first fragment and/or the second fragment is produced, and wherein said first fragment and said second fragment are continuously produced under said isothermal conditions, and (c) detecting the presence of the first fragment, the second fragment, or the first and second fragments, the presence thereof indicating the presence of the DNA analyte.

18. The method of claim 17 wherein the first **oligonucleotide** has a substituent that facilitates separation of the first fragment or the second fragment from the medium.

19. The method of claim 17 wherein first fragment and/or second fragment has a label.

20. The method of claim 19 wherein the label is selected from the group consisting of a member of a specific binding pair, dyes, fluorescent molecules, chemiluminescers, coenzymes, enzyme substrates, radioactive groups and suspendible particles.

21. The method of claim 17 wherein the second **oligonucleotide** hybridizes to the DNA analyte at a site contiguous with the site on the DNA analyte at which the first **oligonucleotide** reversibly hybridizes.

22. The method of claim 17 wherein the first **oligonucleotide** and/or the second **oligonucleotide** is DNA.

23. A method for detecting a polynucleotide analyte, the method comprising: (a) providing in combination a medium suspected of containing the polynucleotide analyte, a first DNA **oligonucleotide** at least a portion of which reversibly hybridizes with the polynucleotide analyte under isothermal conditions, a 5'-nuclease, and a second DNA **oligonucleotide** that hybridizes to a site on the polynucleotide analyte that is 3' of, and contiguous with, the site at which the first DNA **oligonucleotide**, reversibly hybridizes, wherein the polynucleotide analyte is substantially fully hybridized to the second DNA **oligonucleotide** under the isothermal conditions, (b) reversibly hybridizing under the isothermal conditions the polynucleotide analyte and the first DNA **oligonucleotide**, wherein the first DNA **oligonucleotide**, when hybridized to the polynucleotide analyte, is cleaved by the 5'-nuclease as a result of the presence of the polynucleotide analyte to provide, in at least a 100-fold molar excess of the polynucleotide analyte, (i) a first fragment that is substantially non-hybridizable to the polynucleotide analyte and/or (ii) a second fragment that is 3' of the first fragment with reference to the intact first DNA **oligonucleotide** and is substantially hybridizable to the polynucleotide analyte, wherein said first fragment and/or said second fragment is/are continuously produced under said isothermal conditions, and (c) detecting the presence of the first fragment, the second fragment, or the first and second fragments, the presence thereof indicating the presence of the polynucleotide analyte.

24. The method of claim 23 wherein the first fragment and/or the second fragment has a label.

25. The method of claim 24 wherein the label is selected from the group consisting of a member of a specific binding pair, dyes, fluorescent molecules, chemiluminescers, coenzymes, enzyme substrates, radioactive groups and suspendible particles.

26. The method of claim 23 wherein the polynucleotide analyte is DNA.

incubating the **oligonucleotide** with a polynucleotide and a 5'-nuclease under isothermal conditions, wherein at least a portion of the **oligonucleotide** reversibly hybridizes to the polynucleotide under said isothermal conditions and wherein the **oligonucleotide**, when the portion is hybridized to the polynucleotide, is cleaved by the 5'-nuclease to provide (i) a first fragment that is substantially non-hybridizable to the polynucleotide and includes no more than five nucleotides from the 5'-end of the portion and (ii) a second fragment that is 3' of the first fragment with reference to the intact **oligonucleotide** and is substantially hybridizable to the polynucleotide, thereby modifying said **oligonucleotide**, wherein said first fragment and said second fragment are continuously produced under said isothermal conditions.

28. A method for producing **oligonucleotide** cleavage products from an enzyme catalyzed cleavage of the **oligonucleotide**, the method comprising: (a) combining, in any order, a polynucleotide, an **oligonucleotide** having a 3'-portion which substantially hybridizes with the polynucleotide and a 5' portion which does not substantially hybridize with the polynucleotide, and a nuclease, wherein the **oligonucleotide**, when hybridized to the polynucleotide, forms a polynucleotide complex comprising at least the polynucleotide and the **oligonucleotide**, the complex serving as a substrate for the nuclease, (b) incubating the **oligonucleotide**, the polynucleotide and the nuclease at a temperature at which the **oligonucleotide** reversibly hybridizes to the polynucleotide, wherein the nuclease cleaves the **oligonucleotide** when the **oligonucleotide** is hybridized to the polynucleotide to continuously produce at said temperature (i) a first fragment that is substantially non-hybridizable to the polynucleotide and includes no more than 5 nucleotides from the 5'-end of the portion which substantially hybridizes to the polynucleotide, and (ii) a second fragment that is 3' of the first fragment with reference to the intact **oligonucleotide** and is substantially hybridizable to the polynucleotide, thereby producing **oligonucleotide** cleavage products.

29. A method for detecting a polynucleotide analyte, which comprises: (a) reversibly hybridizing an **oligonucleotide** with a polynucleotide analyte and a 5'-nuclease under isothermal conditions wherein the polynucleotide analyte serves as a recognition element to enable the 5'-nuclease to cleave the **oligonucleotide** to provide (i) a first fragment that is substantially non-hybridizable to the polynucleotide analyte, and (ii) a second fragment that lies 3' of the first fragment with reference to the intact **oligonucleotide** and is substantially hybridizable to the polynucleotide analyte, wherein at least a 100-fold molar excess of the first fragment and/or the second fragment are obtained relative to the molar amount of the polynucleotide analyte, wherein said first fragment and said second fragment are continuously produced under said isothermal conditions, and (b) detecting the presence of the first fragment, the second fragment, or the first and second fragments, the presence thereof indicating the presence of the polynucleotide analyte.

L5 ANSWER 92 OF 112 USPATFULL on STN

2000:113705 Oligonucleotide modification, signal amplification, and nucleic acid detection by target-catalyzed product formation.

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US 6110677 20000829

APPLICATION: US 1998-15949 19980130 (9)

DOCUMENT TYPE: Utility; Granted.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method is disclosed for modifying an oligonucleotide, which method has application to the detection of a polynucleotide analyte. An oligonucleotide is reversibly hybridized with a polynucleotide, for example, a polynucleotide analyte, in the presence of a 5'-nuclease under isothermal conditions. The polynucleotide analyte serves as a recognition element to enable a 5'-nuclease to cleave the oligonucleotide to provide (i) a first fragment that is substantially non-hybridizable to the polynucleotide analyte and (ii) a second fragment that lies 3' of the first fragment (in the intact oligonucleotide) and is substantially hybridizable to the polynucleotide analyte. At least a 100-fold molar excess of the first fragment and/or the second fragment are obtained relative to the molar amount of the polynucleotide analyte. The presence of the first fragment and/or the second fragment is detected, the presence thereof indicating the presence of the polynucleotide analyte. The method has particular application to the detection of a polynucleotide analyte such as DNA. Kits for conducting methods in accordance with the present invention are

What is claimed is:

1. A method for modifying an **oligonucleotide**, said method comprising: (a) combining said **oligonucleotide** with a polynucleotide and a 5'-nuclease, said **oligonucleotide** having a 3' portion capable of reversibly hybridizing to said polynucleotide and a 5'-portion which does not hybridize to the polynucleotide, (b) incubating said **oligonucleotide**, said polynucleotide and said nuclease under isothermal conditions, whereby a duplex formed by hybridization of the 3' portion of the **oligonucleotide** to the polynucleotide is in equilibrium with unhybridized **oligonucleotide** and unhybridized polynucleotide, said isothermal conditions being at or near the melting temperature of said complex, and (c) while maintaining said isothermal conditions, cleaving said **oligonucleotide** with said nuclease when said 3'-portion is hybridized to said polynucleotide to provide: (i) a first fragment including said 5'-portion and no more than one nucleotide from the 5'-end of said 3'-portion, and (ii) a second fragment that is 3' of said first fragment with reference to the intact **oligonucleotide**, thereby modifying said **oligonucleotide**, wherein said first fragment and said second fragment are continuously produced under said isothermal conditions.

2. The method of claim 1, wherein the amounts of fragments that are formed are at least 100-fold larger than the amount of said polynucleotide.

3. The method of claim 1, further comprising incubating a second **oligonucleotide** under said isothermal conditions with said **oligonucleotide**, said polynucleotide, and said 5'-nuclease, wherein said second **oligonucleotide** substantially non-reversibly hybridizes under said isothermal conditions to a site on said polynucleotide that is in the 3' direction from the site at which said **oligonucleotide** hybridizes.

4. The method according to claim 3, wherein the melting temperature of the second **oligonucleotide** when hybridized to the polynucleotide is at least 3° C. higher than the melting temperature of the first **oligonucleotide** when hybridized to the polynucleotide.

5. A method for amplifying a signal associated with the presence of a polynucleotide analyte, said method comprising: (a) providing in combination a polynucleotide analyte, a 5'-nuclease and a molar excess, relative to the concentration of said polynucleotide analyte, of an **oligonucleotide** having a 3' portion capable of reversibly hybridizing to said polynucleotide and a 5'-portion which does not hybridize to said polynucleotide, (b) under isothermal conditions, establishing an equilibrium between said **oligonucleotide**, said polynucleotide analyte, and a duplex formed by the hybridization of the 3' portion of said **oligonucleotide** with said polynucleotide analyte, said isothermal conditions being at or near the melting temperature of said duplex, (c) while maintaining said isothermal conditions, cleaving said **oligonucleotide** with said 5'-nuclease when said **oligonucleotide** is hybridized to said polynucleotide to provide, (i) a first fragment including said 5'-portion and no more than one nucleotide from the 5'-end of said 3'-portion, and (ii) a second fragment including at least one of said 3' portion and said 3' portion lacking one nucleotide, wherein at least one of said first fragment and said second fragment generates a signal, and (d) while maintaining said isothermal conditions, maintaining said equilibrium to amplify the amount of at least one of said first fragment and said second fragment and thereby amplifying said signal, wherein said first fragment and said second fragment are continuously produced under said isothermal conditions.

6. The method of claim 5 further comprising maintaining said equilibrium until at least a 100-fold molar excess of said first fragment and/or said second fragment are obtained relative to the molar amount of said polynucleotide analyte.

7. The method of claim 5 wherein said polynucleotide analyte is from a source selected from the group consisting of Corynebacteria, Pneumococci, Streptococci, Staphylococci, Neisseria, Enterobacteriaceae, Enteric bacilli, Hemophilus-Bordetella, Pasteurellae, Brucellae, Aerobic Spore-forming Bacilli, Anaerobic Spore-forming Bacilli, Mycobacteria, Actinomycetes, Spirochetes, Trypanosomes, Mycoplasmas, Listeria monocytogenes, Erysipelothrix rhusiopathiae, Streptobacillus moniliformis, Donovanian granulomatis, Bartonella bacilliformis, Rickettsiae, Adenoviruses, Herpes Viruses, Pox Viruses, Picornaviruses, Myxoviruses, Arboviruses, Reoviruses, Retroviruses, Fungi, Hepatitis Viruses, and Tumor Viruses.

8. The method of claim 5, further comprising hybridizing a second **oligonucleotide** to said polynucleotide analyte under said isothermal conditions, wherein said second **oligonucleotide** hybridizes to a site

at which said **oligonucleotide** hybridizes, and wherein the melting temperature of the second **oligonucleotide** when hybridized to the polynucleotide is at least 3° C. higher than the melting temperature of the first **oligonucleotide** when hybridized to the polynucleotide.

9. The method of claim 5, wherein said **oligonucleotide** hybridization sites are contiguous.

10. The method of claim 5, wherein at least one of said first fragment and said second fragment has a label.

11. The method of claim 10, wherein said label is selected from the group consisting of a member of a specific binding pair, dyes, fluorescent molecules, chemiluminescers, coenzymes, enzyme substrates, radioactive groups, and suspendible particles.

12. A method for detecting a polynucleotide analyte, said method comprising: (a) providing in combination a medium suspected of containing said polynucleotide analyte, a molar excess, relative to the suspected concentration of said polynucleotide analyte, of a first **oligonucleotide** having a 3' portion capable of reversibly hybridizing to said polynucleotide and a 5'-portion which does not hybridize to said polynucleotide, a 5'-nuclease, and a second **oligonucleotide** that hybridizes to a site on said polynucleotide analyte in the 3'-direction of the site at which said first **oligonucleotide** hybridizes, (b) under isothermal conditions, establishing an equilibrium between a complex formed by the hybridization of the 3' portion of said first **oligonucleotide** and said polynucleotide analyte, said polynucleotide analyte and said first **oligonucleotide**, said isothermal conditions being at or near the melting temperature of said complex, and wherein said second **oligonucleotide** is substantially fully hybridized to said polynucleotide analyte under said isothermal conditions, (c) while maintaining said isothermal conditions, cleaving said first **oligonucleotide** when hybridized to said polynucleotide analyte with said 5'-nuclease to provide, (i) a first fragment that is substantially non-hybridizable to said polynucleotide analyte, and (ii) a second fragment that is 3' of said first fragment in said first **oligonucleotide** and which substantially hybridizes to said polynucleotide analyte; and (d) while maintaining said isothermal conditions, detecting the presence of said first fragment, said second fragment, or said first fragment and said second fragment, the presence thereof indicating the presence of said polynucleotide analyte wherein said first fragment and said second fragment are continuously produced under said isothermal conditions.

13. The method according to claim 12, wherein the melting temperature of the second **oligonucleotide** when hybridized to the polynucleotide is at least 3° C. higher than the melting temperature of the first **oligonucleotide** when hybridized to the polynucleotide.

14. The method of claim 12, wherein said first fragment and/or said second fragment has a label.

15. The method of claim 14, wherein said label is selected from the group consisting of a member of a specific binding pair, dyes, fluorescent molecules, chemiluminescers, coenzymes, enzyme substrates, radioactive groups, and suspendible particles.

16. The method of claim 12 wherein said polynucleotide analyte is DNA.

17. The method of claim 12, wherein said first fragment includes no more than 1 nucleotide from the 5'-end of that portion of said first **oligonucleotide** that is capable of hybridizing to said polynucleotide analyte.

18. The method of claim 12, wherein said second **oligonucleotide** hybridizes to said polynucleotide at a site contiguous with the site on said polynucleotide at which said first **oligonucleotide** hybridizes.

19. The method of claim 12, wherein said first **oligonucleotide** has a substituent that facilitates separation of said first fragment or said second fragment from said medium.

20. The method of claim 12 wherein said polynucleotide analyte is from a source selected from the group consisting of Corynebacteria, Pneumococci, Streptococci, Staphylococci, Neisseria, Enterobacteriaceae, Enteric bacilli, Hemophilus-Bordetella, Pasteurellae, Brucellae, Aerobic Spore-forming Bacilli, Anaerobic Spore-forming Bacilli, Mycobacteria, Actinomycetes, Spirochetes, Trypanosomes, Mycoplasmas, Listeria monocytogenes, Erysipelothrix rhusiopathiae, Streptobacillus moniliformis, Donovania granulomatis, Bartonella bacilliformis,

Myxoviruses, Arboviruses, Reoviruses, Retroviruses, Fungi, Hepatitis Viruses, and Tumor Viruses.

21. A method for detecting a polynucleotide analyte, said method comprising: (a) providing in combination a medium suspected of containing said polynucleotide analyte, a first **oligonucleotide** at least a portion of which reversibly hybridizes with said polynucleotide analyte under isothermal conditions to form a complex, said isothermal conditions being at or near the melting temperature of said complex, a 5'-nuclease, and a second **oligonucleotide** that hybridizes to a site on said polynucleotide analyte that is in 3' of, and contiguous with, the site at which said first **oligonucleotide** hybridizes, wherein the melting temperature of the second **oligonucleotide** when hybridized to the polynucleotide is at least 3° C. higher than the melting temperature of the first **oligonucleotide** when hybridized to the polynucleotide, (b) reversibly hybridizing under said isothermal conditions said polynucleotide analyte and said first **oligonucleotide**, wherein said first **oligonucleotide**, when hybridized to said polynucleotide analyte, is cleaved by said 5'-nuclease as a function of the presence of said polynucleotide analyte to provide, in at least a 100-fold molar excess of said polynucleotide analyte, (i) a first fragment that is substantially non-hybridizable to said polynucleotide analyte, and (ii) a second fragment that is 3' of said first fragment in said first **oligonucleotide** and which substantially hybridizes to said polynucleotide analyte; and (c) detecting the presence of said first fragment, said second fragment, or said first fragment and said second fragment, the presence thereof indicating the presence of said polynucleotide analyte, wherein said polynucleotide analyte is from a source selected from the group consisting of Corynebacteria, Pneumococci, Streptococci, Staphylococci, Neisseria, Enterobacteriaceae, Enteric bacilli, Hemophilus-Bordetella, Pasteurellae, Brucellae, Aerobic Spore-forming Bacilli, Anaerobic Spore-forming Bacilli, Mycobacteria, Actinomycetes, Spirochetes, Trypanosomes, Mycoplasmas, Listeria monocytogenes, Erysipelothrix rhusiopathiae, Streptobacillus moniliformis, Donovanella granulomatis, Bartonella bacilliformis, Rickettsiae, Adenoviruses, Herpes Viruses, Pox Viruses, Picornaviruses, Myxoviruses, Arboviruses, Reoviruses, Retroviruses, Fungi, Hepatitis Viruses, and Tumor Viruses.

22. The method of claim 21, wherein at least one of said first fragment and said second fragment has a label.

23. The method of claim 22, wherein said label is selected from the group consisting of a member of a specific binding pair, dyes, fluorescent molecules, chemiluminescers, coenzymes, enzyme substrates, radioactive groups, and suspendible particles.

24. The method of claim 22, wherein said polynucleotide analyte is DNA.

L5 ANSWER 93 OF 112 USPTAFULL on STN

2000:74082 NANBV diagnostics: polynucleotides useful for screening for hepatitis C virus.

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APPLICATION: US 1994-307273 19940916 (8)

DOCUMENT TYPE: Utility; Granted.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A family of cDNA sequences derived from hepatitis C virus (HCV) are provided. These sequences encode antigens which react immunologically with antibodies present in individuals with non-A non-B hepatitis (NANBH), but which are absent from individuals infected with hepatitis A virus, or hepatitis B virus, and also are absent in control individuals. The HCV cDNA sequences lack substantial homology to the sequences of hepatitis delta virus (HDV) and HBV. A comparison of the sequences of amino acids encoded in the HCV cDNA with the sequences of Flaviviruses indicates that HCV may be related to the Flaviviruses. The HCV cDNA sequences and the polypeptides encoded therein are useful as reagents for the detection and therapy of HCV. The reagents provided in the invention are also useful for the isolation of NANBH agent(s), for the propagation of these agents in tissue culture, and for the screening of antiviral agents for HCV.

CLM What is claimed is:

1. A purified preparation of an **oligonucleotide**, wherein the **oligonucleotide** is present in an amount capable of selectively and detectably hybridizing to the genome of a hepatitis C virus (HCV) or its complement relative to other viral agents, and further wherein the **oligonucleotide** comprises a contiguous sequence of at least 8 nucleotides complementary to the genome of an HCV or its complement.

2. The purified preparation of claim 1 wherein the contiguous sequence is at least 10 nucleotides.
3. The purified preparation of claim 1 wherein the contiguous sequence is at least 12 nucleotides.
4. The purified preparation of claim 1 wherein the contiguous sequence is at least 15 nucleotides.
5. The purified preparation of claim 1 wherein the contiguous sequence is at least 20 nucleotides.
6. The purified preparation of claim 1 wherein the **oligonucleotide** is a **primer** for a DNA polymerase or a reverse transcriptase.
7. The purified preparation of claim 1 wherein the **oligonucleotide** is capable of selectively hybridizing to the genome of an HCV.
8. The purified preparation of claim 2 wherein the **oligonucleotide** is capable of selectively hybridizing to the genome of an HCV.
9. The purified preparation of claim 3 wherein the **oligonucleotide** is capable of selectively hybridizing to the genome of an HCV.
10. The purified preparation of claim 4 wherein the **oligonucleotide** is capable of selectively hybridizing to the genome of an HCV.
11. The purified preparation of claim 5 wherein the **oligonucleotide** is capable of selectively hybridizing to the genome of an HCV.
12. The purified preparation of claim 1 wherein the **oligonucleotide** comprises a contiguous sequence of at least 8 nucleotides complementary to either strand of the nucleotide residue sequence depicted in FIG. 62.
13. The purified preparation of claim 2 wherein the **oligonucleotide** comprises a contiguous sequence of at least 10 nucleotides complementary to either strand of the nucleotide residue sequence depicted in FIG. 62.
14. The purified preparation of claim 3 wherein the **oligonucleotide** comprises a contiguous sequence of at least 12 nucleotides complementary to either strand of the nucleotide residue sequence depicted in FIG. 62.
15. A kit for analyzing samples for the presence of HCV comprising an **oligonucleotide** capable of selectively and detectably hybridizing to the genome of a hepatitis C virus (HCV) or its complement relative to other viral agents, wherein the **oligonucleotide** is in a suitable package and comprises a contiguous sequence of at least 8 nucleotides complementary to the genome of an HCV or its complement.
16. The kit of claim 15 wherein the contiguous sequence is at least 10 nucleotides.
17. The kit of claim 15 wherein the contiguous sequence is at least 12 nucleotides.
18. The kit of claim 15 wherein the contiguous sequence is at least 15 nucleotides.
19. The kit of claim 15 wherein the contiguous sequence is at least 20 nucleotides.
20. The kit of claim 15 wherein the **oligonucleotide** is a **primer** for a DNA polymerase or a reverse transcriptase.
21. The kit of claim 15 wherein the **oligonucleotide** is capable of selectively hybridizing to the genome of an HCV.
22. The kit of claim 16 wherein the **oligonucleotide** is capable of selectively hybridizing to the genome of an HCV.
23. The kit of claim 17 wherein the **oligonucleotide** is capable of selectively hybridizing to the genome of an HCV.
24. The kit of claim 18 wherein the **oligonucleotide** is capable of selectively hybridizing to the genome of an HCV.
25. The kit of claim 19 wherein the **oligonucleotide** is capable of selectively hybridizing to the genome of an HCV.
26. The kit of claim 15 wherein the **oligonucleotide** comprises a contiguous sequence of at least 8 nucleotides complementary to either strand of the nucleotide residue sequence depicted in FIG. 62.

27. The kit of claim 16 wherein the **oligonucleotide** comprises a contiguous sequence of at least 10 nucleotides complementary to either strand of the nucleotide residue sequence depicted in FIG. 62.
28. The kit of claim 17 wherein the **oligonucleotide** comprises a contiguous sequence of at least 12 nucleotides complementary to either strand of the nucleotide residue sequence depicted in FIG. 62.
29. A purified preparation of a polynucleotide encoding a polypeptide comprising a contiguous sequence of at least 10 amino acids encoded by the genome of HCV and comprising an antigenic determinant.
30. The purified preparation according to claim 29 wherein said contiguous sequence is at least 15 amino acids.
31. The purified preparation according to claim 29 wherein said contiguous sequence is from amino acids 1 to 2955 of the HCV polyprotein.
32. The purified preparation according to claim 29 wherein said contiguous sequence is encoded within the lambda-gt11 cDNA library deposited with the American Type Culture Collection (ATCC) under accession no. 40394.
33. The purified preparation according to claim 29 wherein said contiguous sequence is from a nonstructural viral protein.
34. The purified preparation according to claim 29 wherein said contiguous sequence is from a structural viral protein.
35. The purified preparation according to claim 29 which is a DNA polynucleotide.
36. The purified preparation a polynucleotide according to claim 29 which is an RNA polynucleotide.
37. A recombinant polynucleotide vector comprising a polynucleotide sequence encoding a polypeptide comprising a contiguous sequence of at least 10 amino acids from an HCV polyprotein, wherein said polypeptide comprises an antigenic determinant.
38. A vector according to claim 37 wherein said contiguous sequence is from amino acids 1 to 3011 of the HCV polyprotein.
39. A vector according to claim 37 capable of expressing the polypeptide encoded by said DNA sequence.
40. A vector according to claim 37 which is a DNA vector.
41. A vector according to claim 37 which is an RNA vector.
42. A host cell transformed by a recombinant vector according to claim 37 wherein a coding sequence is operably linked to a control sequence capable of providing for expression of the coding sequence by the host cell.

L5 ANSWER 94 OF 112 USPATFULL on STN

2000:24448 Method for introducing defined sequences at the 3' end of polynucleotides.

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US 6030774 20000229

APPLICATION: US 1995-479745 19950607 (8)

DOCUMENT TYPE: Utility; Granted.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method is disclosed for extending a primer to produce a single stranded polydeoxynucleotide that has two or more defined sequences. A combination is provided which comprises a template polynucleotide, a blocker polynucleotide, a primer polynucleotide and a polynucleotide Q. The template polynucleotide has three sequences T1, T2 and T3 wherein T1 is non-contiguous and 3' of T3 and wherein the 5' end of T3 is 5' of the 5' end of T2. The primer polynucleotide has a second defined sequence at its 3' end that is hybridizable with T1. The blocker polynucleotide has sequence B1 that is hybridizable with T3. Polynucleotide Q has sequences S1 and S2 wherein S1 is 3' of S2 and homologous with T2 and S2 is complementary to a first defined sequence that is to be introduced at the 3' end of the polynucleotide primer, when it is extended during the

end of the blocker polynucleotide or present as a separate reagent. The primer is extended along the template polynucleotide and along at least a portion of sequence T2 and thereafter along the polynucleotide Q to give a single stranded polynucleotide having two or more defined sequences.

CLM What is claimed is:

1. A method for detecting the presence of a polynucleotide analyte, said analyte comprising a template sequence having three sequences T1, T2 and T3 wherein T1 is non-contiguous with and 3' of said T2 and T3 and the 5' end of said T3 is 5' of the 5' end of said T2, in a medium suspected of containing said analyte said method comprising: (a) combining said medium with (1) a **primer** polynucleotide whose 3' end is hybridizable with said T1, (2) a blocker polynucleotide with sequence B1, said B1 being hybridizable with said T3, (3) a polynucleotide Q having sequences S1 and S2 wherein Q is attached at its 3'-end to the 5' end of said blocker polynucleotide or is present as a separate reagent and wherein S1 is 3' of S2 and is substantially identical to said T2 and wherein said S2 is substantially identical to at least the 3' end of said **primer** polynucleotide, (4) DNA polymerase and (5) deoxynucleoside triphosphates under conditions wherein: (A) said blocker becomes hybridized to said template, (B) said **primer** becomes hybridized with and extended along, by means of a polymerase, said template and along at least a portion of said T2 and thereafter along said polynucleotide Q to form a duplex, wherein said polymerase has little or no 5'-3' exonuclease activity under said conditions for extending and wherein the 3'-end of said polynucleotide Q, when present as a separate reagent, is not extended by said polymerase, (C) said extended **primer** is dissociated from the said duplex, and (D) said **primer** hybridizes with and is extended along said extended **primer** to form a duplex comprising extended **primer** and steps (C) and (D) are repeated, and (b) examining for the presence of said extended **primer**.

2. The method of claim 1 wherein at least a five base sequence within the 15 bases at the 3' end of said T3 is comprised of at least 80% G and C nucleotides.

3. The method of claim 1 wherein said S1 is from 5 to 50 nucleotides in length.

4. The method of claim 1 wherein the 5' end of said S1 is complementary to the 5' end of said B1.

5. The method of claim 1 wherein the 5' end of said S1 not complementary to the 5' end of said B1.

6. The method of claim 1 wherein said T2 homologous is contiguous with said T3.

7. The method of claim 1 wherein said T2 is not contiguous with said T3.

8. The method of claim 1 wherein said polynucleotide analyte is DNA.

9. The method of claim 1 wherein said polynucleotide is RNA and said medium includes reverse transcriptase.

10. The method of claim 1 wherein said polydeoxynucleotide **primer** is labeled with a reporter molecule.

11. The method of claim 1 wherein said polynucleotide Q is attached to the 5' end of said blocker polynucleotide.

12. A method for detecting the presence of a polynucleotide analyte comprising a template sequence having three sequences T1, T2 and T3 wherein T1 is non-contiguous with and 3' of said T2 and T3 and the 3' end of said T3 is contiguous with or lies within said T2, in a medium suspected of containing said analyte, said method comprising: (a) combining said medium with (1) a **primer** polynucleotide whose 3' end is hybridizable with said T1, (2) a blocker polynucleotide with sequence B1, said B1 being hybridizable with said T3, (3) a polynucleotide Q having sequences S1 and S2 wherein Q is attached at its 3'-end to the 5' end of said blocker polynucleotide and wherein S1 is 3' of said S2 and is substantially identical to said T2 and wherein said S2 is substantially identical to at least the 3' end of said **primer** polynucleotide, (4) DNA polymerase and (5) deoxynucleoside triphosphates under conditions wherein: (A) said blocker becomes hybridized to said template, (B) said **primer** becomes hybridized with and extended along, by means of a polymerase, said template polynucleotide and along at least a portion of said T2 and thereafter along said polynucleotide Q to form a duplex, wherein said polymerase has little or no 5'-3' exonuclease activity under said conditions for extending, (C) the extended **primer** is dissociated from the said duplex, and (D) said **primer** hybridizes with and is extended along said extended **primer** to

repeated, and (b) examining for the presence of said extended **primer**.

13. The method of claim 12 wherein at least a five base sequence within the 15 bases at the 3' end of said T3 is comprised of at least 80% G and C nucleotides.

14. The method of claim 12 wherein said S1 is from 5 to 50 nucleotides in length.

15. The method of claim 12 wherein the 5' end of said S1 is complementary to the 5' end of said B1.

16. The method of claim 12 wherein the 5' end of said S1 not complementary to the 5' end of said B1.

17. The method of claim 12 wherein said T2 is contiguous with said T3.

18. The method of claim 12 wherein said polynucleotide analyte is DNA.

19. The method of claim 12 wherein said polynucleotide is RNA and said medium includes reverse transcriptase.

20. The method of claim 12 wherein said polydeoxynucleotide **primer** is labeled with a reporter molecule.

L5 ANSWER 95 OF 112 USPATEFULL on STN

1999:128348 Isothermal transcription based assay for the detection and genotyping of **dengue virus**.

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US 5968732 19991019

APPLICATION: US 1997-2177 19971231 (9)

DOCUMENT TYPE: Utility; Granted.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB An isothermal transcription based amplification assay for **dengue virus** RNA uses primer combinations for sequences within the envelope gene or the 3' non-coding region of the virus and a probe. Probes may be specific for a serotype of **dengue virus**.

CLM What is claimed is:

1. A method for the detection of **dengue virus** RNA, comprising: a) obtaining a sample which may contain **dengue virus** RNA; b) performing an isothermal transcription based amplification on the sample with two **primers**, a first **primer** selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, and SEQ ID NO:21, and a second **primer** selected from the group consisting of SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:8 and SEQ ID NO:9; and c) detecting the amplification product using a labeled probe, wherein the probe is selected from the group consisting of SEQ ID NO:5 and SEQ ID NO:10, whereby hybridization of the probe to the amplification product indicates the presence of **dengue virus** RNA in the sample.

2. The method of claim 1, wherein the sample comprises cells or virus and RNA is extracted from the cells or virus in the sample prior to step b).

3. A method for the detection or genotyping of **dengue virus** RNA in a sample comprising: a) obtaining a sample which may contain **dengue virus** RNA; b) performing an isothermal transcription based amplification on the sample with two **primers**, a first **primer** selected from the group consisting of SEQ ID NO:11 and SEQ ID NO:22, and a second **primer** containing SEQ ID NO:12; and c) detecting the amplification product using a probe, wherein the probe is selected from the group consisting of SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16 and SEQ ID NO:17, whereby hybridization of the amplification product with SEQ ID NO:13 indicates the virus RNA is type 1, hybridization of the amplification product with SEQ ID NO:14 indicates the virus RNA is type 2, hybridization of the amplification product with SEQ ID NO:15 indicates the virus RNA is type 3, hybridization of the amplification product with SEQ ID NO:16 indicates the virus RNA is type 4, and wherein hybridization of the amplification product with SEQ ID NO:17 indicates the presence of **dengue virus** RNA.

4. The method of claim 3, wherein the sample comprises cells or virus and RNA is extracted from the cells or virus in the sample prior to step b).

5. A kit for the detection or genotyping of **dengue virus** RNA in a sample, comprising two **primers**, a first **primer** containing an

SEQ ID NO:22, and a second **primer** containing SEQ ID NO:12, and at least one probe, wherein the at least one probe is selected from the group consisting of SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16 and SEQ ID NO:17.

6. A kit for the detection of **dengue virus** type 2 RNA in a sample, said kit comprising two **primers**, a first **primer** containing an oligonucleotide selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:20, and SEQ ID NO:21, and a second **primer** containing an **oligonucleotide** selected from the group consisting of SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:8, and SEQ ID NO:9; and a probe, wherein the probe is selected from the group consisting of SEQ ID NO:5, SEQ ID NO:10, and SEQ ID NO:23.

7. An **oligonucleotide** selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:20, SEQ ID NO: 21, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, and SEQ ID NO:23.

8. An **oligonucleotide** selected from the group consisting of SEQ ID NO: 11, SEQ ID NO:20, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17.

L5 ANSWER 96 OF 112 USPATFULL on STN

1999:96205 Methods and reagents for rapid diagnosis of **dengue virus** infection

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APPLICATION: US 1997-840344 19970428 (8)

DOCUMENT TYPE: Utility; Granted.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Specific primers that amplify a portion of the 3'-noncoding regions of **dengue virus** types 1, 2, 3 and 4, and a method of using these primers in a rapid reverse transcriptase-**polymerase chain reaction** (RT-PCR) for specific detection of dengue viruses, but not other flaviviruses, is disclosed.

CLM What is claimed is:

1. A method of detecting **dengue virus** and not Japanese encephalitis virus in a biological sample, said method comprising the steps of: incubating RNA extracted from the sample with reverse transcriptase and a first **dengue virus**-specific **primer** including at least 15 consecutive nucleotides of SEQ ID NO:3, wherein the first **dengue virus**-specific **primer** is complementary to a portion of the 3' noncoding region in the dengue viral nucleic acid, for a time and under conditions sufficient to allow double stranded nucleic acid to form; adding a second **dengue virus**-specific **primer** including at least 15 consecutive nucleotides of SEQ ID NO:1, wherein the second **dengue virus**-specific **primer** is identical to a portion of the 3' noncoding region in the dengue viral nucleic acid, and a thermostable DNA polymerase; incubating for a time and under conditions sufficient to allow said double stranded nucleic acid, if any, to be amplified by **polymerase chain reaction** to form reaction products; and detecting the reaction products as an indication of the presence of **dengue virus** and not Japanese encephalitis virus in the sample.

2. The method of claim 1, wherein a third **dengue virus**-specific **primer** is added along with the second **primer**, wherein the third **primer** includes at least 15 consecutive nucleotides of SEQ ID NO:2, wherein the second **dengue virus**-specific **primer** is identical to a portion of the 3' noncoding region in the dengue viral nucleic acid.

3. A method of claim 2, wherein the third **primer** consists of the nucleotide sequence of SEQ ID NO:2.

4. A method of claim 1, wherein the first **primer** consists of the nucleotide sequence of SEQ ID NO:3.

5. A method of claim 1, wherein the second **primer** consists of the nucleotide sequence of SEQ ID NO:1.

6. A kit for detecting **dengue virus** and not Japanese encephalitis virus in a sample, the kit comprising: a first **dengue virus**-specific **primer** including at least 15 consecutive nucleotides of SEQ ID NO:3, wherein the first **dengue virus**-specific **primer** is complementary to a portion of the 3' noncoding region in the dengue viral nucleic acid; a second **dengue virus**-specific **primer** including at least 15

virus-specific primer is identical to a portion of the 3' noncoding region in the dengue viral nucleic acid; and reagents for performing reverse-transcriptase-**polymerase chain reaction** (RT-PCR).

7. The kit of claim 6, further comprising a third **dengue virus-specific primer** including at least 15 consecutive nucleotides of SEQ ID NO:2, wherein the second **dengue virus-specific primer** is identical to a portion of the 3' noncoding region in the dengue viral nucleic acid.

8. A kit of claim 7, wherein the third **primer** consists of the nucleotide sequence of SEQ NO:2.

9. A kit of claim 6, wherein the first **primer** consists of the nucleotide sequence of SEQ ID NO:3.

10. A kit of claim 6, wherein the second **primer** consists of the nucleotide sequence of SEQ ID NO:1.

11. An isolated nucleic acid including at least 15 consecutive nucleotides of SEQ ID NO:3, wherein the nucleic acid is complementary to a portion of the 3' noncoding region in the dengue viral nucleic acid.

12. The nucleic acid of claim 11 consisting of the nucleotide sequence of SEQ ID NO:3.

13. An isolated nucleic acid including at least 15 consecutive nucleotides of SEQ ID NO:1, wherein the nucleic acid is identical to a portion of the 3' noncoding region in the dengue viral nucleic acid.

14. The nucleic acid of claim 13 consisting of the nucleotide sequence of SEQ ID NO:1.

15. An isolated nucleic acid including at least 15 consecutive nucleotides of SEQ ID NO:2, wherein the nucleic acid is identical to a portion of the 3' noncoding region in the dengue viral nucleic acid.

16. The nucleic acid of claim 15 consisting of the nucleotide sequence of SEQ ID NO:2.

17. An isolated nucleic acid including at least 15 consecutive nucleotides of SEQ ID NO:6, wherein the nucleic acid is identical to a portion of the 3' noncoding region in the dengue viral nucleic acid.

18. The nucleic acid of claim 17 consisting of the nucleotide sequence of SEQ ID NO:6.

L5 ANSWER 97 OF 112 USPATFULL on STN

1999:69620 Homogeneous amplification and detection of nucleic acids.

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US 5914230 19990622

APPLICATION: US 1996-771624 19961220 (8)

PRIORITY: US 1995-9090P 19951222 (60)

DOCUMENT TYPE: Utility; Granted.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to a method for detecting or amplifying and detecting a target polynucleotide sequence. The method comprises providing in combination (i) a medium suspected of containing the target polynucleotide sequence, (ii) all reagents required for conducting an amplification of the target polynucleotide sequence when amplification is desired, and (iii) two oligonucleotide probes capable of binding to a single strand of the product of the amplification. At least one of the probes has two sequences that either (i) are non-contiguous and bind to contiguous or non-contiguous sites on the single strand or (ii) can bind to non-contiguous sites on the single strand. Each probe may contain a label. The combination is subjected to conditions for amplifying the target polynucleotide sequence. Next, the combination is subjected to conditions under which both of the probes hybridize to one of the strands to form a termolecular complex, which is detected by means of the label.

CLM What is claimed is:

1. A method for amplifying and detecting a target polynucleotide sequence, which comprises: (a) providing in combination (i) a medium suspected of containing said target polynucleotide sequence, (ii) all reagents required for conducting an amplification of said target

bind to a single strand of the product of said amplification wherein at least one of said probes has two sequences, which are each about 8 to 25 nucleotides in length and which either (i) are non-contiguous and can bind to contiguous or noncontiguous sites on said single strand or (ii) can bind to non-contiguous sites on said single strand, (b) subjecting said combination to conditions for amplifying said target polynucleotide sequence, (c) after step (b) subjecting said combination to conditions under which both of said probes hybridize to one of said strands to form a termolecular complex and (d) detecting said complex.

2. The method of claim 1 wherein said reagents comprise two **oligonucleotides** that bind to said target polynucleotide sequence and an enzyme capable of modifying at least one of said **oligonucleotides** as a function of the presence of said target polynucleotide sequence.

3. The method of claim 1 wherein said reagents comprise an **oligonucleotide primer** that binds to and is extended along said target polynucleotide sequence, a nucleotide polymerase and nucleotide triphosphates.

4. The method of claim 1 wherein said reagents comprise (i) two **oligonucleotide primers** that each bind to and are extended along said target polynucleotide sequence and a sequence complementary to said target polynucleotide sequence, respectively, (ii) a nucleotide polymerase and (iii) nucleotide triphosphates.

5. The method of claim 1 wherein each probe contains a label and said combination further comprises particles that bind to said labels and said detecting comprises detecting agglutination of said particles.

6. A method for amplifying and detecting a target polynucleotide sequence of a polynucleotide analyte, which comprises: (a) providing in combination a sample suspected of containing a polynucleotide analyte having said target polynucleotide sequence, reagents for amplifying said polynucleotide analyte to produce copies of said target polynucleotide sequence, a first **oligonucleotide** probe having nucleotide sequences S1 and S2 and a second **oligonucleotide** probe having sequences S3 and S4 wherein each of S1, S2, S3 and S4 is about 8 to 25 nucleotides in length and wherein the sequences comprising at least one of the probes are linked such that either (i) they are non-contiguous and can bind to contiguous or non-contiguous sites on one of the strands of said copies or (ii) the sites to which they hybridize on one of the strands of said copies are noncontiguous and wherein said probes (i) do not hybridize to said copies during said amplifying and (ii) subsequent to said amplifying, both of said probes can hybridize to one of the strands of said copies, and (iv) each of said probes is comprised of a label that facilitates detection of said probes hybridized to said strands, (b) subjecting said combination to conditions for amplifying said polynucleotide analyte, (c) after step (b) subjecting said combination to conditions under which both of said probes hybridize to one of said strands to form a termolecular complex, and (d) detecting said complex.

7. The method of claim 6 wherein the 3'-terminus of said S1 is linked to the 5'-terminus of said S2 by a linking group comprising a chain of 1 to 200 atoms.

8. The method of claim 7 wherein said chain comprises from 1 to 40 nucleotides or nucleotide analogs.

9. The method of claim 7 wherein at least a portion of said linking group is an **oligonucleotide** label.

10. The method of claim 6 wherein the 3'-terminus of said S1 is linked to the 5'-terminus of said S2 by a linking group and the 3'-terminus of said S3 is linked to the 5'-terminus of said S4 by a linking group, each of said linking groups comprising from 0 to 40 nucleotides or nucleotide analogs.

11. The method of claim 6 wherein detection of said complex comprises association of said complex with particles.

12. The method of claim 11 wherein said detection comprises detecting agglutination of said particles.

13. The method of claim 6 wherein said label is an **oligonucleotide** label.

14. The method of claim 6 wherein said S1 hybridizes to a site on said copies that lies 5' of the hybridization site of said S2 on said copy wherein the 3'-end of said S1 is linked to the 5'-end of said S2.

15. The method of claim 6 wherein S1 and S2 are linked by a nucleotide

16. The method of claim 15 wherein each of said linking groups comprises an **oligonucleotide** label.

17. The method of claim 16 wherein said combination comprises **oligonucleotides** N1 and N2 that are each respectively complementary to one of said **oligonucleotide** labels wherein said N1 and N2 are each labeled with a reporter group.

18. The method of claim 17 wherein said N1 is associated with a particle having a photosensitizer associated therewith and said N2 is associated with a particle having a chemiluminescent compound associated therewith.

19. A method of detecting a target polynucleotide containing a target polynucleotide sequence wherein all reagents required for said method are first combined with said target polynucleotide, said method comprising: (a) dissociating said target polynucleotide sequence into single strands when said target polynucleotide sequence is double stranded, (b) hybridizing an **oligonucleotide primer** to the 3'-end of each of said single strands, (c) extending said **primer** hybridized to each of said single strands along the single strand to produce a copy of said target polynucleotide sequence, (d) dissociating said copy into single strands, (e) hybridizing two **oligonucleotide** probes to one of said single strands wherein at least one of said probes is comprised of two sequences that hybridize with one of said single strands, wherein said sequences either (i) are non-contiguous and can bind to contiguous or non-contiguous sites on said strand or (ii) the sites on said strand to which said sequences hybridize are non-contiguous, (f) detecting the binding of both of said probes to said single strand, the presence thereof being related to the presence of said target polynucleotide.

20. The method of claim 19 wherein each of said probes is comprised of a label.

21. The method of claim 19 wherein at least one of said probes is bound to or can become bound to a particle.

22. The method of claim 21 wherein said particle is comprised of a photosensitizer.

23. The method of claim 21 wherein said particle is comprised of a luminescent compound.

24. The method of claim 19 wherein each of said probes is comprised of two sequences that hybridize with said single strand, said sequences and/or said sites on said strand to which said sequences hybridize being non-contiguous.

25. The method of claim 19 wherein each of said probes is comprised of an **oligonucleotide** label.

26. The method of claim 25 wherein said detecting comprises binding of nucleotides N1 and N2, respectively, to said **oligonucleotide** label of each of said probes wherein said N1 is bound to a first label and said N2 is bound to a second label.

27. The method of claim 26 wherein said first label is comprised of a photosensitizer.

28. A method for detecting a target sequence of a target polynucleotide ("target sequence"), said method comprising: (a) amplifying said target sequence by a method comprising: (i) hybridizing to the 3'-end of said target sequence a first **oligonucleotide primer** ("first **primer**"), (ii) extending, in the presence of a polymerase and nucleotide triphosphates, said first **primer** along at least said target sequence to produce an extended first **primer**, said first **primer** hybridizing to, and being extended along, (1) said extended first **primer** or (2) an extended second **oligonucleotide primer** ("second **primer**") wherein said extended second **primer** results from the extension of a second **primer** that hybridizes to and is extended along a polynucleotide that is complementary (complementary polynucleotide) to said target sequence, (iii) dissociating said extended first **primer** from said target sequence, (iv) hybridizing, to the 3'-end of said extended first **primer**, said first or said second **primer**, (v) extending said first or said second **primer** along said extended first **primer**, (vi) dissociating said extended first **primer** or said extended second **primer** from said extended first **primer**, (vii) hybridizing, to the 3'-end of said extended first or said extended second **primer**, said first **primer**, and (viii) repeating steps (v)-(vii), and (b) detecting said extended first **primer** and/or said extended second **primer** by means of a first **oligonucleotide** probe having nucleotide sequences S1 and S2 and a second **oligonucleotide** probe having sequences S3 and S4,

wherein the sequences comprising at least one of the probes are linked such that its two sequences either (i) are non-contiguous and can bind to contiguous or non-contiguous sites on one of said extended **primers** or (ii) hybridize to sites on one of said extended **primers** that are noncontiguous and wherein said probes (A) are present during said amplifying, (B) do not hybridize to said extended first and/or second **primers** during said amplifying of step (a) and (C) do not interfere with said amplifying of step (a) and (D) subsequent to said amplifying of step (a), both of said probes can hybridize to one of said extended first and/or said extended second **primers** to form a termolecular complex, and (E) one or both of said probes contains a label that facilitates detection of said probes hybridized to said extended first and/or said extended second **primers**.

29. The method of claim 28 wherein the repeating of steps (v)-(vii) is achieved by repeated temperature cycling.

30. The method of claim 28 wherein said target polynucleotide is DNA.

31. The method of claim 28 wherein only said first **primer** is used and said target sequence contains at its 5'- end at least a 10-base sequence hybridizable with a sequence at the 3' end of said target sequence to which said first **primer** hybridizes.

32. The method of claim 28 wherein said first and said second **primers** are different and said extended first **primer** is a template for said second **primer** and said extended second **primer** is a template for said first **primer**.

33. The method of claim 28 wherein the 3'-terminus of said S1 is linked to the 5'-terminus of said S2 by a linking group comprising from 0 to 40 nucleotides or nucleotide analogs.

34. The method of claim 28 wherein the 3'-terminus of said S1 is linked to the 5'-terminus of said S2 by a linking group and the 3'-terminus of said S3 is linked to the 5'-terminus of said S4 by a linking group, each of said linking groups comprising from 0 to 40 nucleotides or nucleotide analogs.

35. The method of claim 28 wherein detection of said complex comprises association of said complex with particles.

36. The method of claim 35 wherein said detection comprises detecting agglutination of said particles.

37. The method of claim 28 wherein said label is an **oligonucleotide** label.

38. The method of claim 28 wherein said sequences comprising at least one of the probes are linked by a linking group, at least a portion of said linking group comprising an **oligonucleotide** label.

39. The method of claim 38 wherein each of said probes comprises an **oligonucleotide** label and said detecting of step (b) comprises the use of nucleotides N1 and N2, each respectively complementary to one of said **oligonucleotide** labels, wherein said N1 and N2 are each labeled with a reporter molecule.

40. The method of claim 39 wherein said N1 is associated with a particle having a photosensitizer associated therewith and said N2 is associated with a particle having a chemiluminescent compound associated therewith.

41. A kit for use in an amplification and detection of a target polynucleotide sequence of a target polynucleotide, said kit comprising in packaged combination: (a) an **oligonucleotide primer** which hybridizes to said target polynucleotide and is extended along said target polynucleotide sequence to produce extended **oligonucleotide primer**, (b) nucleoside triphosphates, (c) a nucleotide polymerase, (d) a first **oligonucleotide** probe having nucleotide sequences S1 and S2, and (e) a second **oligonucleotide** probe having sequences S3 and S4, wherein S1, S2, S3 and S4 are each about 10 to 20 nucleotides in length and wherein the sequences comprising at least one of said first or said second **oligonucleotide** probes are linked such that either (i) they are non-contiguous and can bind to contiguous or non-contiguous sites on said extended polynucleotide **primer** or a complementary sequence thereto or (ii) the sites to which they hybridize on said extended polynucleotide **primer** or a complementary sequence thereto are noncontiguous, and wherein said probes have the characteristics that they (i) do not hybridize to said extended **oligonucleotide primer** during said amplification and (ii) subsequent to said amplification, both of said first and second **oligonucleotide** probes can hybridize to said extended **oligonucleotide primer** or said complementary sequence,

detection of said probes hybridized to said extended **oligonucleotide primer** or said complementary sequence.

42. The kit of claim 41 wherein the 3'-terminus of said S1 is linked to the 5'-terminus of said S2 by a nucleotide linking group.

43. The kit of claim 42 wherein at least a portion of said nucleotide linking group is an **oligonucleotide** label.

44. The kit of claim 41 wherein one of said probes is associated with a particle.

45. The kit of claim 44 wherein said particle is bound to or binds to said probe.

46. The kit of claim 41 wherein said label is an **oligonucleotide** label.

47. The kit of claim 41 wherein S1 and S2 are linked by a nucleotide linking group and S3 and S4 are linked by a nucleotide linking group.

48. The kit of claim 47 wherein said linking groups comprise **oligonucleotide** labels.

49. The kit of claim 48 comprising nucleotide N1 and N2 each complementary to one of said **oligonucleotide** labels wherein said N1 and N2 are each labeled with a reporter molecule.

50. The kit of claim 49 wherein said N1 is associated with a particle having a photosensitizer associated therewith and said N2 is associated with a particle having a chemiluminescent compound associated therewith.

51. The kit of claim 41 comprising a second **oligonucleotide primer**.

52. A kit for detection of a target polynucleotide sequence, said kit comprising in packaged combination reagents for conducting an amplification of said target polynucleotide sequence and two labeled **oligonucleotide** probes that bind to the product of said amplification of said target polynucleotide sequence wherein at least one of said probes has two sequences that are each about 10 to 20 nucleotides in length and that are non-contiguous and can bind to contiguous or non-contiguous sites on a single strand of said product.

53. A method for amplifying and detecting a target polynucleotide sequence, which comprises: (a) providing in combination a sample suspected of containing a target polynucleotide having said target polynucleotide sequence, reagents for amplifying said target polynucleotide sequence to produce copies thereof, a first **oligonucleotide** probe and a second **oligonucleotide** probe wherein said probes (i) do not hybridize to said copies during said amplifying and (ii) do not interfere with said amplifying and (iii) subsequent to said amplifying, both of said probes can hybridize to one the strands of said copies, and wherein at least one said probes is associated with a particle, (b) subjecting said combination to conditions for amplifying said target polynucleotide sequence to produce said copies, (c) thereafter subjecting said combination to conditions under which both of said probes hybridize to one of said strands and result in agglutination of said particles, and (d) detecting said agglutination wherein the presence of agglutination indicates the presence of said target polynucleotide sequence.

L5 ANSWER 98 OF 112 USPATFULL on STN

1999:33776 Detection of nucleic acids by formation of template-dependent product.

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US 5882867 19990316

APPLICATION: US 1995-486301 19950607 (8)

DOCUMENT TYPE: Utility; Granted.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method is disclosed for detecting a target polynucleotide sequence. The method comprises incubating an oligonucleotide with the target polynucleotide sequence and a nucleotide polymerase under isothermal conditions wherein at least one nucleotide is added to the 3'-terminus of the oligonucleotide to provide an extended oligonucleotide having the additional nucleotides. The presence of extended oligonucleotide is detected as an indication of the presence of the target polynucleotide sequence. The method has particular application to the detection of DNA.

CLM What is claimed is:

comprises: (a) reversibly hybridizing an **oligonucleotide** with a target polynucleotide sequence in the presence of a nucleotide polymerase and 1 to 3 different nucleoside triphosphates under isothermal conditions wherein said target polynucleotide sequence serves as a template for addition of at least one nucleotide to the 3'-terminus of said **oligonucleotide** under said isothermal conditions to provide an extended **oligonucleotide** wherein at least a 100-fold molar excess of said extended **oligonucleotide** is obtained relative to the molar amount of said target polynucleotide sequence and wherein said isothermal conditions comprise an isothermal temperature within 15° C. below and 15° C. above the melting temperature of the **oligonucleotide**:target polynucleotide sequence complex and wherein said isothermal conditions exclude fluctuation in temperature referred to as thermal cycling, and (b) detecting the presence of said extended **oligonucleotide**, the presence thereof indicating the presence of said target polynucleotide sequence.

2. The method of claim 1 wherein at least said nucleotide has a label.

3. The method of claim 1 wherein said **oligonucleotide** has a label.

4. The method of claim 1 wherein only 1 nucleotide is added.

5. The method of claim 1 wherein said target polynucleotide sequence is DNA.

6. A method for detecting a target polynucleotide sequence, said method comprising: (a) providing in combination a medium suspected of containing said target polynucleotide sequence, a molar excess, relative to the suspected concentration of said target polynucleotide sequence, of an **oligonucleotide** capable of hybridizing with said target polynucleotide sequence, 1 to 3 different nucleoside triphosphates and a nucleotide polymerase, (b) reversibly hybridizing under isothermal conditions said target polynucleotide sequence and said **oligonucleotide**, wherein said **oligonucleotide** is extended under said isothermal conditions at its 3-end to produce an amount of an extended **oligonucleotide** that is at least 100 times the molar amount of said target polynucleotide sequence, wherein said isothermal conditions comprise an isothermal temperature within 15° C. below and 15° C. above the melting temperature of the **oligonucleotide**:target polynucleotide sequence complex and wherein said isothermal temperature is a constant temperature with a variance of $\pm 2^\circ$ C., and (c) detecting the presence of said extended **oligonucleotide**, the presence thereof indicating the presence of said target polynucleotide sequence.

7. The method of claim 6 wherein at least one of said nucleoside triphosphates has a label.

8. The method of claim 7 wherein said **oligonucleotide** has a label.

9. The method of claim 6 wherein only 1 nucleoside triphosphate is added.

10. The method of claim 7 wherein said label is selected from the group consisting of members of specific binding pairs, dyes, fluorescent molecules, chemiluminescers, coenzymes, enzyme substrates and radioactive groups.

11. The method of claim 6 wherein said target polynucleotide sequence is DNA.

12. The method of claim 6 wherein said nucleoside triphosphates are selected from the group consisting of dUTP, dITP, dATP, dCTP and dGTP and dideoxynucleoside triphosphates.

13. A method for detecting a DNA analyte, said method comprising: (a) providing in combination a medium suspected of containing said DNA analyte, an **oligonucleotide** capable of hybridizing with said DNA analyte, 1 to 3 different nucleoside triphosphates and a template dependent DNA polymerase, (b) reversibly hybridizing said DNA analyte and said **oligonucleotide** under isothermal conditions, wherein said **oligonucleotide** is extended at its 3-terminus under said isothermal conditions to produce at least a 100-fold excess, relative to said DNA analyte, of an extended **oligonucleotide**, wherein said isothermal conditions comprise an isothermal temperature within 15° C. below and 15° C. above the melting temperature of the **oligonucleotide**:DNA analyte complex and wherein said isothermal temperature is a constant temperature with a variance of $\pm 2^\circ$ C. and (c) detecting the presence of said extended **oligonucleotide**, the presence thereof indicating the presence of said DNA analyte.

substituent that facilitates separation of said **oligonucleotide** from said medium.

15. The method of claim 13 wherein said **oligonucleotide** has a label.

16. The method of claim 13 wherein at least one of said nucleoside triphosphates has a label.

17. The method of claim 16 wherein said label is selected from the group consisting of members of specific binding pairs, dyes, fluorescent molecules, chemiluminescers, coenzymes, enzyme substrates, radioactive groups and small organic molecules.

18. The method of claim 15 wherein said label is selected from the group consisting of members of specific binding pairs, dyes, fluorescent molecules, chemiluminescers, coenzymes, enzyme substrates, radioactive groups and small organic molecules.

19. A method for detecting the presence of a target polynucleotide sequence, said method comprising: (a) forming, in relation to the presence of said target polynucleotide sequence, at least a 100-fold excess, relative to said target polynucleotide sequence, of an extended **oligonucleotide** having at least two labels wherein during said forming an **oligonucleotide** is reversibly hybridized under isothermal conditions with said target polynucleotide sequence in the presence of a nucleotide polymerase and 1 to 3 different nucleoside triphosphates, wherein said target polynucleotide sequence serves as a template for addition of at least one nucleotide to the 3-end of said **oligonucleotide** under said isothermal conditions to provide an extended **oligonucleotide** and wherein one of said labels is part of said nucleotide and the other of said labels is part of said **oligonucleotide** and wherein said isothermal conditions comprise an isothermal temperature within 15° C. below and 15° C. above the melting temperature of the **oligonucleotide**:target polynucleotide sequence complex and wherein said isothermal conditions exclude fluctuation in temperature referred to as thermal cycling and (b) detecting both of said labels as an indication of the presence of said **oligonucleotide**, the presence thereof indicating the presence of said target polynucleotide sequence.

20. A method of forming an **oligonucleotide** having at least two labels, said method comprising the steps of: (a) providing in combination a catalytic amount of a target polynucleotide, a nucleotide polymerase, a first-labeled deoxynucleoside triphosphate, and a second-labeled **oligonucleotide** that is complementary to at least a portion of said target polynucleotide, (b) treating said combination under isothermal conditions such that said second-labeled **oligonucleotide** reversibly hybridizes to said target polynucleotide to form a duplex and said first-labeled deoxynucleoside triphosphate becomes linked to said labeled **oligonucleotide** under said isothermal conditions, wherein said isothermal conditions comprise an isothermal temperature within 15° C. below and 15° C. above the melting temperature of the **oligonucleotide**:target polynucleotide complex and wherein said isothermal temperature is a constant temperature with a variance of $\pm 2^\circ$ C.

21. A method for detecting a mutation in a target polynucleotide sequence, which comprises the steps of: (a) reversibly hybridizing an **oligonucleotide** with a target polynucleotide sequence suspected of having said mutation in the presence of a nucleotide polymerase under isothermal conditions in the presence of a nucleotide polymerase and 1 to 3 different nucleoside triphosphates wherein said target polynucleotide sequence serves as a template for addition of at least one nucleotide to the 3'-terminus of said **oligonucleotide** under said isothermal conditions to provide an extended **oligonucleotide** wherein at least a 100-fold molar excess of said extended **oligonucleotide** is obtained relative to the molar amount of said target polynucleotide sequence, wherein said nucleotide contains a label and wherein said isothermal conditions comprise an isothermal temperature within 15° C. below and 15° C. above the melting temperature of the **oligonucleotide**:target polynucleotide sequence complex and wherein said isothermal temperature is a constant temperature with a variance of $\pm 2^\circ$ C. and (b) detecting the presence of said label in said extended **oligonucleotide**, the presence thereof indicating the presence of said mutation in said target polynucleotide sequence.

22. The method of claim 21 wherein said target polynucleotide sequence is DNA.

23. The method of claim 21 wherein one nucleotide is added to said **oligonucleotide**, said nucleotide being complementary to the nucleotide suspected of being mutated.

1999:33768 Internal positive controls for nucleic acid amplification.

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US 5882857 19990316

APPLICATION: US 1995-475283 19950607 (8)

DOCUMENT TYPE: Utility; Granted.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to an improvement in a method for amplifying a target sequence of a target polynucleotide. The method comprises combining a sample suspected of containing the target polynucleotide with reagents for amplifying the target sequence and subjecting the combination to conditions wherein the target sequence is amplified. The present improvement comprises including in the combination a control oligonucleotide and a control polynucleotide that has a sequence that is hybridizable with the control oligonucleotide. When the control oligonucleotide is bound to the control polynucleotide, the ability of a primer to chain extend along the control polynucleotide is reduced. Optionally, the control oligonucleotide is part of the control polynucleotide. The method finds particular application in the area of nucleic acid amplification and detection.

CLM What is claimed is:

1. In a method for forming multiple copies of a target sequence of a target polynucleotide, said method comprising the step of forming extension products of a first **oligonucleotide primer** capable of specifically hybridizing to, and being extended at least along, said target sequence or along an extended first **oligonucleotide primer**, said extension products being copies of said target sequence, the improvement which comprises forming said extension products in the presence of a control **oligonucleotide** and a control polynucleotide wherein said control polynucleotide has a subsequence that is specifically hybridizable with said control **oligonucleotide** and wherein said control **oligonucleotide** when bound to said control polynucleotide reduces the ability of a second **oligonucleotide primer**, which may be the same as or different from said first **oligonucleotide primer** and which specifically hybridizes to said control polynucleotide, to chain extend along said control polynucleotide and wherein said control **oligonucleotide** is optionally part of said control polynucleotide.
2. The method of claim 1 wherein said subsequence lies outside a second sequence of said control polynucleotide wherein said second sequence is hybridizable with said second **oligonucleotide primer**.
3. The method of claim 1 wherein said subsequence lies within a second sequence of said control polynucleotide wherein said second sequence is hybridizable with said second **oligonucleotide primer**.
4. The method of claim 3 wherein said subsequence lies at the 5'-end of said second sequence.
5. The method of claim 1 wherein said first **oligonucleotide primer** and said second **oligonucleotide primer** are the same.
6. The method of claim 1 wherein the presence of amplified target sequence is detected, the presence of said amplified target sequence being an indication of the presence of said target polynucleotide.
7. A method for forming multiple copies of a target sequence of a single stranded target polynucleotide in a reaction mixture, said method comprising: (a) hybridizing to a 3'-end of said target sequence a first **oligonucleotide primer** wherein said target sequence comprises a subsequence that has the same sequence as the sequence of the first **oligonucleotide primer** or comprises a subsequence that has the same sequence as that of a second **oligonucleotide primer**, (b) extending said first **oligonucleotide primer** along at least said target sequence to form an extended first **oligonucleotide primer** sequence, (c) dissociating said extended first **oligonucleotide primer** sequence from said target sequence, (d) hybridizing, to a 3'-end of said extended first **oligonucleotide primer** sequence, said first or said second **oligonucleotide primer** wherein said first or said second **oligonucleotide primer** is capable of hybridizing to, and extending along, said extended first **oligonucleotide primer** sequence, (e) extending said first or said second **oligonucleotide primer** along said extended first **oligonucleotide primer** sequence to form a sequence complementary to said extended first **oligonucleotide primer** sequence, (f) dissociating said complementary sequence from said extended first **oligonucleotide primer** sequence, (g) hybridizing, to

primer, and (h) repeating steps (e)-(g), the improvement comprising including, in said reaction mixture subjected to steps (a)-(g) above, a control **oligonucleotide** and a control polynucleotide that includes a subsequence that is specifically hybridizable with said control **oligonucleotide** and a subsequence that is specifically hybridizable with said first **oligonucleotide primer** or said second **oligonucleotide primer**, wherein said control **oligonucleotide** when hybridized to said control polynucleotide reduces the ability of said first **oligonucleotide primer** or said second **oligonucleotide primer** to chain extend along said control polynucleotide and wherein said control **oligonucleotide** is optionally part of said control polynucleotide.

8. The method of claim 7 wherein the subsequence of the control polynucleotide to which the control **oligonucleotide** hybridizes lies outside the subsequence to which said first **oligonucleotide primer** or said second **oligonucleotide primer** hybridizes.

9. The method of claim 7 wherein, the subsequence of the control polynucleotide to which the control **oligonucleotide** hybridizes lies within the subsequence to which said first **oligonucleotide primer** or said second **oligonucleotide primer** hybridizes.

10. The method of claim 9 wherein the subsequence of the control polynucleotide to which the control **oligonucleotide** hybridizes lies at the 5'-end of the subsequence to which said first **oligonucleotide primer** or said second **oligonucleotide primer** hybridizes.

11. The method of claim 7 wherein the presence of said copies of said target sequence are detected, the presence thereof being an indication of the presence of said target polynucleotide.

12. The method of claim 7 wherein said control oligo nucleotide is 5 to 30 nucleotides in length.

13. The method of claim 7 wherein said control **oligonucleotide** is present in the reaction mixture at a concentration of from about 1 nM to 100 uM.

14. The method of claim 7 wherein said control **oligonucleotide** is part of said control polynucleotide and is located within 5 to 300 nucleotides of said subsequence.

15. The method of claim 7 wherein the repeating of steps (e)-(g) is achieved by repeated temperature cycling.

16. The method of claim 15 wherein temperature cycling is repeated at least 3 times.

17. The method of claim 7 wherein said target polynucleotide is DNA.

18. The method of claim 7 wherein said extending is carried out in the presence of nucleoside triphosphates and nucleotide polymerase.

19. The method of claim 7 wherein only said first **primer** is used and said target sequence contains at its 5' end at least a 10 base sequence hybridizable with a sequence at the 3' end of said target sequence to which said first **primer** hybridizes.

20. The method of claim 7 wherein said first and second **primers** are different and extended first **primer** is a template for said second **primer** and said extended second **primer** is a template for said first **primer**.

21. A method for forming multiple copies of at least one double stranded polynucleotide, said polynucleotide comprising a single stranded target polynucleotide sequence and its complementary sequence, said method having a positive internal control, said method comprising: (a) treating a sample suspected of containing one or more of said double stranded polynucleotides with (i) **oligonucleotide primers** capable of specifically hybridizing to a portion of each target polynucleotide sequence and its complementary sequence suspected of being present in said sample under conditions for hybridizing said **oligonucleotide primers** to and extending said **oligonucleotide primers** along said target polynucleotide sequence and said complementary sequence, wherein said **oligonucleotide primers** are selected such that the extension product formed from one **oligonucleotide primer**, when it is dissociated from its complement, can serve as a template for the formation of the extension product of another **oligonucleotide primer**, (ii) a control **oligonucleotide**, and (iii) a control polynucleotide that is amplifiable by at least one of the same **oligonucleotide primers** as said target polynucleotide sequence and

oligonucleotide wherein said control **oligonucleotide** when bound to said control polynucleotide reduces the ability of said at least one of the same **oligonucleotide primers** to chain extend along said control polynucleotide and wherein said control **oligonucleotide** is optionally part of said control polynucleotide, said conditions allowing for said control **oligonucleotide** to hybridize to said control polynucleotide so as to cause a reduced efficiency of amplification of the control polynucleotide relative to the efficiency of said amplification in the absence of said control **oligonucleotide**, (b) dissociating **oligonucleotide primer** extension products from their templates if the target polynucleotide sequence is present to produce single stranded molecules and (c) treating the single stranded molecules produced in step (b) with said **oligonucleotide primers** of step (a) under conditions such that an **oligonucleotide primer** extension product is formed using the single strands produced in step (b) as templates, resulting in amplification of the target polynucleotide sequence and complementary sequence if present, said conditions allowing for the extension of said same **oligonucleotide primer** along said control polynucleotide.

22. The method of claim 21 wherein said subsequence lies outside a second sequence of said control polynucleotide wherein said second sequence is hybridizable with said same **oligonucleotide primer**.

23. The method of claim 21 wherein said subsequence lies within a second sequence of said control polynucleotide wherein said second sequence is specifically hybridizable with said same **oligonucleotide primer**.

24. The method of claim 23 wherein said subsequence lies at the 5'-end of said second sequence.

25. The method of claim 21 wherein the presence of said **oligonucleotide primer** extension products are detected, the presence thereof indicating the presence of said polynucleotide.

26. The method of claim 21 wherein said control oligo nucleotide is 5 to 30 nucleotides in length.

27. The method of claim 21 wherein said control **oligonucleotide** is present in the reaction mixture at a concentration of from about 1 nM to 100 uM.

28. The method of claim 21 wherein said control **oligonucleotide** is part of said control polynucleotide and is located within 5 to 300 nucleotides of said subsequence.

29. The method of claim 21 wherein said conditions of step (c) are achieved by repeated temperature cycling.

30. The method of claim 29 wherein temperature cycling is repeated at least 3 times.

31. The method of claim 21 wherein said polynucleotide is DNA.

32. The method of claim 21 wherein said extending is carried out in the presence of nucleoside triphosphates and nucleotide polymerase.

33. The method of claim 21 comprising adding to the product of step (c) a labeled **oligonucleotide** probe for each target polynucleotide sequence and complementary sequence being amplified wherein said probe is capable of specifically hybridizing to said target polynucleotide sequence or said complementary sequence or a mutation thereof and determining whether hybridization has occurred.

34. A method of producing multiple copies of a target sequence of a target polynucleotide, which comprises: (a) providing in combination (I) a single stranded polynucleotide having a sequence that is specifically hybridizable with said target sequence and that is flanked at each end by first and second flanking sequences that are specifically hybridizable to one another, (II) an **oligonucleotide primer** at least a 10 base portion of which at its 3'-end is hybridizable to whichever of said first and second flanking sequences is at the 3'-end of said single stranded polynucleotide, (III) nucleoside triphosphates, (IV) nucleotide polymerase, (V) a control **oligonucleotide** and (VI) a control polynucleotide that is amplifiable by the same **primer** as in (II) and has a subsequence that is hybridizable with said control **oligonucleotide** wherein said control **oligonucleotide** when bound to said control polynucleotide reduces the ability of a **primer** to chain extend along said control polynucleotide and wherein said control **oligonucleotide** is optionally part of said control polynucleotide and (b) incubating said combination under conditions for either wholly sequentially or partially sequentially or concomitantly (I) dissociating

(II) hybridizing said **oligonucleotide primer** with the flanking sequence at the 3'-end of said single stranded polynucleotide and with said control polynucleotide and hybridizing said control **oligonucleotide** to said control polynucleotide, (III) extending said **oligonucleotide primer** along said single stranded polynucleotide to provide a first extended **oligonucleotide primer** and extending said **oligonucleotide primer** along said control polynucleotide up to the point of hybridization of said control **oligonucleotide** to said control polynucleotide to provide an extended control **primer**, (IV) dissociating said first extended **primer** and said single stranded polynucleotide and dissociating said control polynucleotide and said control **oligonucleotide** and said control extended **primer**, (V) hybridizing said first extended **oligonucleotide primer** with said **oligonucleotide primer** and hybridizing said **oligonucleotide primer** and said control **oligonucleotide** with said control polynucleotide, (VI) extending said **oligonucleotide primer** along said first extended **oligonucleotide primer** to provide a second extended **oligonucleotide primer** and extending said **oligonucleotide primer** along said control polynucleotide to provide a control extended **oligonucleotide primer**, (VII) dissociating said second extended **oligonucleotide primer** from said first extended **oligonucleotide primer** and said control **oligonucleotide** and said control extended **primer** and said control polynucleotide, and (VII) repeating steps (V)-(VII) above.

35. The method of claim 34 wherein said subsequence lies outside a second sequence of said control polynucleotide wherein said second sequence is hybridizable with said **oligonucleotide primer**.

36. The method of claim 34 wherein said subsequence lies within a second sequence of said control polynucleotide wherein said second sequence is hybridizable with a said **oligonucleotide primer**.

37. The method of claim 36 wherein said subsequence lies at the 5'-end of said second sequence.

38. The method of claim 34 wherein the presence of extended **oligonucleotide primers** are detected, the presence thereof indicating the presence or amount of said target polynucleotide.

39. The method of claim 38 wherein said **oligonucleotide primer** is labeled with a reporter group.

40. The method of claim 34 wherein said control **oligonucleotide** is 5 to 30 nucleotides in length.

41. The method of claim 34 wherein said control **oligonucleotide** is present in the reaction mixture at a concentration of from about 1 nM to 100 μ M.

42. The method of claim 34 wherein said control **oligonucleotide** is part of said control polynucleotide and is located within 5 to 300 nucleotides of said sequence.

43. The method of claim 34 wherein the repeating of steps (e)-(g) is achieved by repeated temperature cycling.

44. The method of claim 43 wherein temperature cycling is repeated at least 3 times.

45. The method of claim 34 wherein said target polynucleotide is DNA.

46. A method for detecting a polynucleotide analyte, said method comprising: (a) hybridizing to the 3'-end of said polynucleotide analyte a first **oligonucleotide primer**, (b) extending said first **oligonucleotide primer** along at least said polynucleotide analyte, said first **oligonucleotide primer** being capable of specifically hybridizing to, and being extended along, (I) said extended first **oligonucleotide primer** wherein said polynucleotide analyte comprises a first sequence that is hybridizable with said first **oligonucleotide primer** and a second sequence that is hybridizable with said first sequence or (II) an extended second **oligonucleotide primer** wherein said extended second **oligonucleotide primer** is produced by the extension of a second **oligonucleotide primer** capable of specifically hybridizing to and extending along a complementary polynucleotide that is complementary to said polynucleotide analyte, (c) dissociating extended first **oligonucleotide primer** from said polynucleotide analyte, (d) hybridizing, to the 3'-end of said extended first **oligonucleotide primer**, said first or said second **oligonucleotide primer**, (e) extending said first or said second **oligonucleotide primer** along said extended first **oligonucleotide primer**, (f) dissociating said extended first **oligonucleotide primer** or said

oligonucleotide primer, (g) hybridizing, to the 3'-end of said extended first or second **oligonucleotide primer**, said first **oligonucleotide primer**, (h) repeating steps (e)-(g), wherein steps (a)-(g) above are conducted in the presence of a control **oligonucleotide** and a control polynucleotide wherein said control polynucleotide has a subsequence that is specifically hybridizable with said control **oligonucleotide** and wherein said control **oligonucleotide** when bound to said control polynucleotide reduces the ability of said first or second **oligonucleotide primer** which specifically hybridizes to said control polynucleotide to chain extend along said control polynucleotide and wherein said control **oligonucleotide** is optionally part of said control polynucleotide, and (i) detecting said extended first and/or second **oligonucleotide primer**, the presence thereof indicating the presence of said polynucleotide analyte.

47. The method of claim 46 wherein said subsequence lies outside a second sequence of said control polynucleotide wherein said second sequence is hybridizable with said first or said second **primer**.

48. The method of claim 46 wherein said subsequence lies within a second sequence of said control polynucleotide wherein said second sequence is hybridizable with said first or said second **primer**.

49. The method of claim 48 wherein said subsequence lies at the 5'-end of said second sequence.

50. The method of claim 46 wherein said control **oligonucleotide** is 5 to 30 nucleotides in length.

51. The method of claim 46 wherein said control **oligonucleotide** is present in the reaction mixture at a concentration of from about 1 nM to 100 uM.

52. The method of claim 46 wherein said control **oligonucleotide** is part of said control polynucleotide and is located within 5 to 300 nucleotides of said sequence.

53. The method of claim 46 wherein the repeating of steps (e)-(g) is achieved by repeated temperature cycling.

54. The method of claim 53 wherein temperature cycling is repeated at least 3 times.

55. The method of claim 46 wherein said polynucleotide analyte is DNA.

56. The method of claim 46 wherein said extending is carried out in the presence of nucleoside triphosphates and nucleotide polymerase.

57. The method of claim 46 wherein only said first **primer** is used and said polynucleotide analyte contains at its 5' end at least a 10 base sequence hybridizable with a sequence at the 3' end of said polynucleotide analyte to which said first **primer** hybridizes.

58. The method of claim 46 wherein said first and second **primers** are different and extended first **primer** is a template for said second **primer** and said extended second **primer** is a template for said first **primer**.

59. In a method for forming multiple copies of a target sequence of a target polynucleotide, said method comprising the step of forming extension products of an **oligonucleotide primer** along said target sequence or along an extended **oligonucleotide primer**, said extension products being copies of said target sequence, the improvement which comprises forming said extension products in the presence of a control **oligonucleotide** and a control polynucleotide wherein said control polynucleotide has a subsequence that is specifically hybridizable with said control **oligonucleotide** and said **oligonucleotide primer**, and wherein said control **oligonucleotide** when bound to said control polynucleotide reduces the ability of said **oligonucleotide primer** to chain extend along said control polynucleotide and wherein said control **oligonucleotide** is optionally part of said control polynucleotide.

60. A kit comprising in packaged combination: (a) a control **oligonucleotide** that is part of a control polynucleotide, which also comprises a sequence (hybridizable sequence) that is hybridizable with said control **oligonucleotide**, said control **oligonucleotide** being non-chain extendable along said control polynucleotide (b) an **oligonucleotide primer** that is hybridizable with said hybridizable sequence of said control polynucleotide, (c) nucleoside triphosphates, and (d) a nucleotide polymerase.

said **oligonucleotide primers** being related in that a product of the extension of one of said first or said second **oligonucleotide primer** along a target sequence serves as a template for the extension of the other of said first or said second **oligonucleotide primer**.

L5 ANSWER 100 OF 112 USPATFULL on STN

1999:30594 Human transaldolase: an autoantigen with a function in metabolism.

Perl, Andras, Jamesville, NY, United States

The Research Foundation of State University of New York, Albany, NY, United States (U.S. corporation)

US 5879909 19990309

APPLICATION: US 1998-57762 19980409

DOCUMENT TYPE: Utility; Granted.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Transaldolase is an enzyme which acts as an autoantigen in immune-related neurodegenerative diseases, particularly multiple sclerosis. Human transaldolase, the DNA coding therefore, peptides derived therefrom, and DNA control elements associated therewith and anti-transaldolase antibodies are disclosed. These compositions are useful in methods such as immunoassays for detecting subjects making anti-transaldolase antibodies and diagnosing the neurodegenerative disease.

CLM What is claimed is:

1. An isolated DNA molecule encoding (i) a human transaldolase protein molecule SEQ ID NO:2, (ii) a peptide fragment thereof or (iii) a functional derivative thereof, or an allelic variant of said transaldolase-encoding DNA molecule, which peptide fragment or functional derivative comprises one or more T cell epitopes or one or more B cell/antibody epitopes, with the proviso that said DNA molecule is not: (a) an **oligonucleotide** of 18 nucleotides encoding Thr-Leu-Leu-Phe-Ser-Phe; Tyr-Asn-Tyr-Tyr-Lys-Lys; or Ala-Asn-Thr-Asp-Lys-Lys; (b) an **oligonucleotide** of 21 nucleotides encoding Asp-Arg-Ile-Leu-Ile-Lys-Leu; Thr-Thr-Val-Val-Ala-Asp-Thr; or Ala-Cys-Ala-Glu-Ala-Gly-Val; (c) an **oligonucleotide** of 24 nucleotides encoding Gln-Ala-Val-Ala-Cys-Ala-Glu-Ala; or Val-Val-Ala-Asp-Thr-Gly-Asp-Phe; (d) an **oligonucleotide** of 27 nucleotides encoding Thr-Thr-Val-Val-Ala-Asp-Thr-Gly-Asp; Thr-Leu-Leu-Phe-Ser-Phe-Ala-Gln-Ala; or Ser-Thr-Glu-Val-Asp-Ala-Arg-Leu-Ser; (e) an **oligonucleotide** of 33 nucleotides encoding Tyr-Lys-Thr-Ile-Val-Met-Gly-Ala-Ser-Phe-Arg; or Thr-Thr-Asn-Pro-Ser-Leu-Ile-Leu-Ala-Ala; (f) an **oligonucleotide** of 36 nucleotides encoding Pro-Gln-Asp-Ala-Thr-Thr-Asn-Pro-Ser-Leu-Ile-Leu; or Leu-Ile-Ser-Pro-Phe-Val-Gly-Arg-Ile-Leu-Asp-Trp; (g) an **oligonucleotide** of 42 nucleotides encoding Val-Thr-Leu-Ile-Ser-Pro-Phe-Val-Gly-Arg-Ile-Leu-Asp-Trp; or Gly-Arg-Val-Ser-Thr-Glu-Val-Asp-Ala-Arg-Leu-Ser-Phe-Asp; or (h) an **oligonucleotide** of 45 nucleotides encoding Pro-Gly-Arg-Val-Ser-Thr-Glu-Val-Asp-Ala-Arg-Leu-Ser-Phe-Asp; and which DNA molecule is substantially free of other human DNA molecules with which it is natively associated.

2. A DNA molecule according to claim 1 which is a cDNA sequence.

3. A DNA molecule according to claim 1 having the nucleotide sequence SEQ ID NO:1.

4. A recombinant DNA molecule according to claim 1 encoding a peptide having the amino acid sequence: (a) residues 1-139 of SEQ ID NO:2, or (b) residues 150-337 of SEQ ID NO:2.

5. A recombinant DNA molecule according to claim 1 encoding a peptide selected from the group consisting of SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21 and SEQ ID NO:22.

6. A recombinant DNA molecule according to claim 1 encoding a peptide or functional derivative which peptide or derivative comprises: (a) one or more T helper cell epitopes each having at least about 8 amino acids (b) one or more cytotoxic T cell epitopes each having between about 8 and 12 amino acids or (c) one or more B cell or antibody epitopes having at least about 6 amino acids.

7. A recombinant DNA molecule according to claim 1 encoding a peptide or functional derivative which peptide or derivative has one or more of the following activities: (a) inhibits a proliferative response of transaldolase-reactive T lymphocytes, (b) inhibits cytotoxicity of transaldolase-reactive cytotoxic T lymphocytes; or (c) inhibits the binding of a transaldolase-specific antibody to transaldolase.

8. A DNA molecule according to claim 1 which is an expression vehicle.

9. The DNA molecule of claim 8, wherein said expression vehicle is a plasmid.

11. A eukaryotic host transfected with a DNA molecule according to claim 8.

12. A DNA molecule which is a promoter or enhancer sequence for the DNA molecule of claim 1, and is selected from the group consisting of SEQ ID NO:23 and SEQ ID NO:24.

13. A process for preparing a human transaldolase protein peptide or a functional derivative thereof, said process comprising: (a) culturing the host of claim 10 which expresses said protein, peptide or functional derivative under culturing conditions; (b) expressing said protein, peptide or functional derivative; and (c) recovering said protein, peptide or functional derivative from said culture.

14. A process for preparing a human transaldolase protein, peptide or a functional derivative thereof, said process comprising: (a) culturing the host of claim 11 which expresses said protein, peptide or functional derivative under culturing conditions; (b) expressing said protein, peptide or functional derivative; and (c) recovering said protein, peptide or functional derivative from said culture.

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(FILE 'HOME' ENTERED AT 22:54:50 ON 03 FEB 2006)

FILE 'USPATFULL' ENTERED AT 22:55:09 ON 03 FEB 2006

E WANG WEI KUNG/IN

L1 12 S E3
L2 1163 S (DENGUE VIRUS)
L3 727 S L2 AND (PCR OR POLYMERASE CHAIN REACTION)
L4 178 S L3 AND (PRIMER?/CLM OR OLIGONUCLEOTIDE?/CLM)
L5 112 S L4 AND AY<2003

=> d 15,cbib,ab,clm,101-112

L5 ANSWER 101 OF 112 USPATFULL on STN

1998:131529 Kits for nucleic acid amplification kit using single primer.

Rose, Samuel, Mountain View, CA, United States

Goodman, Thomas C., Mountain View, CA, United States

Western, Linda M., Mountain View, CA, United States

Becker, Martin, Palo Alto, CA, United States

Ullman, Edwin F., Atherton, CA, United States

Behring Diagnostics GmbH, Deerfield, IL, United States (U.S. corporation)

US 5827649 19981027

APPLICATION: US 1994-242931 19940516 (8)

DOCUMENT TYPE: Utility; Granted.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method is disclosed for determining the presence of a polynucleotide analyte in a sample suspected of containing the analyte. The method comprises (a) forming as a result of the presence of an analyte a single stranded polynucleotide comprising a target polynucleotide binding sequence flanked by first and second polynucleotide sequences that differ from the sequence of the analyte or a sequence complementary to the analyte sequence, (b) forming multiple copies of the single stranded polynucleotide, and (c) detecting the single stranded polynucleotide. Also disclosed is a method of producing at least one copy of a single stranded polynucleotide. The method comprises (a) forming in the presence of nucleoside triphosphates and template dependent polynucleotide polymerase an extension of a polynucleotide primer at least the 3'-end of which has at least a 10 base sequence hybridizable with a second sequence flanking the 3'-end of the single stranded polynucleotide, the second sequence being partially or fully complementary with at least a 10 base first sequence flanking the 5' end of the single stranded polynucleotide, (b) dissociating the extended polynucleotide primer and the single stranded polynucleotide, (c) repeating step a and (d) dissociating the extended polynucleotide primer and the copy of the single stranded polynucleotide.

CLM What is claimed is:

1. A kit for use in determining a polynucleotide analyte, which comprises in packaged combination: a first polynucleotide probe having a first nucleotide sequence capable of hybridizing to a first portion of said polynucleotide analyte, a second polynucleotide probe having a second nucleotide sequence capable of hybridizing to a second portion of the same strand of said polynucleotide analyte other than with the portion recognized by said first nucleotide sequence of said first polynucleotide probe, said first polynucleotide probe and said second polynucleotide probe each comprising a sequence of nucleotides that are hybridizable with one another when said first nucleotide sequence and said second nucleotide sequence are hybridized to their respective

nucleotides being located in its respective polynucleotide probe at or near the end thereof opposite the site of said first nucleotide sequence or said second nucleotide sequence, respectively, means for covalently attaching said first and second polynucleotide sequences when said first and second polynucleotide probes are hybridized with said polynucleotide analyte thereby forming covalently attached first and second polynucleotide sequences such that a polynucleotide **primer** that hybridizes to and is extended along said covalently attached sequences forms an extension product comprising said first and second polynucleotide sequences, a polynucleotide **primer** capable of hybridizing with said second polynucleotide probe and of being extended along said covalently attached sequences in the direction of said first polynucleotide probe, and means for extending said polynucleotide **primer**, said means comprising a polynucleotide polymerase and one or more deoxynucleoside triphosphates.

2. The kit of claim 1 further comprising: a ligase as a means for covalently attaching said first and second nucleotide sequences.

3. The kit of claim 1 wherein said first polynucleotide probe has, of having, means for immobilizing said probe.

4. The kit of claim 1 wherein at least one of said first or second probe contains a polynucleotide sequence that serves as a label.

5. The kit of claim 1 wherein said first and second portions of said polynucleotide analyte are not contiguous.

L5 ANSWER 102 OF 112 USPATFULL on STN

1998:95391 Detection of nucleic acids by target-catalyzed product formation.

Western, Linda M., San Mateo, CA, United States

Rose, Samuel J., Los Altos, CA, United States

Ullman, Edwin F., Atherton, CA, United States

Dade Behring Marburg GmbH, Deerfield, IL, United States (U.S. corporation)

US 5792614 19980811

APPLICATION: US 1996-691627 19960802 (8)

DOCUMENT TYPE: Utility; Granted.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method is disclosed for modifying an oligonucleotide, which method has application to the detection of a polynucleotide analyte. An oligonucleotide is reversibly hybridized with a polynucleotide, for example, a polynucleotide analyte, in the presence of a 5'-nuclease under isothermal conditions. The polynucleotide analyte serves as a recognition element to enable a 5'-nuclease to cleave the oligonucleotide to provide (i) a first fragment that is substantially non-hybridizable to the polynucleotide analyte and (ii) a second fragment that lies 3' of the first fragment (in the intact oligonucleotide) and is substantially hybridizable to the polynucleotide analyte. At least a 100-fold molar excess of the first fragment and/or the second fragment are obtained relative to the molar amount of the polynucleotide analyte. The presence of the first fragment and/or the second fragment is detected, the presence thereof indicating the presence of the polynucleotide analyte. The method has particular application to the detection of a polynucleotide analyte such as DNA. Kits for conducting methods in accordance with the present invention are also disclosed.

CLM What is claimed is:

1. A method for modifying an **oligonucleotide**, said method comprising incubating said **oligonucleotide** with a polynucleotide a single nucleoside triphosphate and a 5'-nuclease wherein at least a portion of said **oligonucleotide** is reversibly hybridized to said polynucleotide under isothermal conditions to form a complex, said isothermal conditions being at or near the melting temperature of said complex, wherein said **oligonucleotide** is present in an amount in excess of the amount of said polynucleotide and wherein said **oligonucleotide**, when said portion is hybridized to said polynucleotide, is cleaved by said 5'-nuclease to provide (i) a first fragment that is substantially non-hybridizable to said polynucleotide and includes no more than one nucleotide from the 5'-end of said portion and (ii) a second fragment that is 3' of said first fragment with reference to the intact **oligonucleotide** and is substantially hybridizable to said polynucleotide.

2. The method of claim 1 wherein the amounts of fragments that are formed are at least 100-fold larger than the amount of said polynucleotide.

3. The method of claim 1 wherein a second **oligonucleotide** is present during said incubating, said second **oligonucleotide** having the characteristic of hybridizing to a site on said polynucleotide that is 3' of the site at which said **oligonucleotide** is reversibly hybridized

polynucleotide under said isothermal conditions.

4. The method of claim 3 wherein said second **oligonucleotide** hybridizes to said polynucleotide at a site contiguous with the site on said polynucleotide at which said first **oligonucleotide** reversibly hybridizes.

5. The method of claim 4 wherein the amounts of fragments that are formed are at least 100-fold larger than the amount of said polynucleotide.

6. A method for detecting a polynucleotide analyte, which comprises: (a) reversibly hybridizing an **oligonucleotide** with a polynucleotide analyte in the presence of a single nucleoside triphosphate and a 5'-nuclease under isothermal conditions wherein said polynucleotide analyte serves as a recognition element to enable said 5'-nuclease to cleave said **oligonucleotide** to provide (i) a first fragment that is substantially non-hybridizable to said polynucleotide analyte and (ii) a second fragment that lies 3' of said first fragment in the intact **oligonucleotide** and is substantially hybridizable to said polynucleotide analyte wherein at least a 100-fold molar excess of said first fragment and/or said second fragment are obtained relative to the molar amount of said polynucleotide analyte, and (b) detecting the presence of said first fragment and/or said second fragment, the presence thereof indicating the presence of said polynucleotide analyte.

7. The method of claim 6 wherein at least one of said first fragment and said second fragment has a label.

8. The method of claim 6 wherein said first fragment includes no more than 1 nucleotide from the 5'-end of that portion of said **oligonucleotide** that hybridizes to said polynucleotide analyte.

9. The method of claim 6 wherein a second **oligonucleotide** is present during said reversible hybridizing, said second **oligonucleotide** having the characteristic of hybridizing to a site on said polynucleotide analyte that is 3' of the site at which said **oligonucleotide** hybridizes wherein said polynucleotide analyte is substantially fully hybridized to said second **oligonucleotide** under said isothermal conditions.

10. The method of claim 9 wherein said **oligonucleotide** hybridization sites are contiguous.

11. A method for detecting a polynucleotide analyte, said method comprising: (a) providing in combination a medium suspected of containing said polynucleotide analyte, a molar excess, relative to the suspected concentration of said polynucleotide analyte, of a first **oligonucleotide** at least a portion of which is capable of reversibly hybridizing with said polynucleotide analyte under isothermal conditions to form a complex, said isothermal conditions being at or near the melting temperature of said complex, a 5'-nuclease, a single nucleoside triphosphate and a second **oligonucleotide** having the characteristic of hybridizing to a site on said polynucleotide analyte that is 3' of the site at which said first **oligonucleotide** hybridizes wherein said polynucleotide analyte is substantially fully hybridized to said second **oligonucleotide** under said isothermal conditions, (b) reversibly hybridizing under said isothermal conditions said polynucleotide analyte and said first **oligonucleotide**, wherein said first **oligonucleotide**, when hybridized to said polynucleotide analyte, is cleaved by said 5'-nuclease as a function of the presence of said polynucleotide analyte to provide, in at least a 100-fold molar excess of said polynucleotide analyte, (i) a first fragment that is substantially non-hybridizable to said polynucleotide analyte and (ii) a second fragment that is 3' of said first fragment in said first **oligonucleotide** and is substantially hybridizable to said polynucleotide analyte, and (c) detecting the presence of said first fragment and/or said second fragment, the presence thereof indicating the presence of said polynucleotide analyte.

12. The method of claim 11 wherein said first fragment and/or said second fragment has a label.

13. The method of claim 12 wherein said label is selected from the group consisting of a member of a specific binding pair, dyes, fluorescent molecules, chemiluminescers, coenzymes, enzyme substrates, radioactive groups and suspendible particles.

14. The method of claim 11 wherein said polynucleotide analyte is DNA.

15. The method of claim 11 wherein said first fragment includes no more than 1 nucleotide from the 5'-end of that portion of said first **oligonucleotide** that is capable of hybridizing to said polynucleotide

16. The method of claim 11 wherein said second **oligonucleotide** hybridizes to said polynucleotide at a site contiguous with the site on said polynucleotide at which said first **oligonucleotide** hybridizes.

17. A method for detecting a DNA analyte, said method comprising: (a) providing in combination a medium suspected of containing said DNA analyte, a first **oligonucleotide** at least a portion of which is capable of reversibly hybridizing with said DNA analyte under isothermal conditions to form a complex, said isothermal conditions being at or near the melting temperature of said complex, a 5'-nuclease, a single nucleoside triphosphate and a second **oligonucleotide** having the characteristic of hybridizing to a site on said DNA analyte that is 3' of the site at which said first **oligonucleotide** hybridizes wherein said DNA analyte is substantially fully hybridized to said second **oligonucleotide** under said isothermal conditions, (b) reversibly hybridizing said DNA analyte and said first **oligonucleotide** under said isothermal conditions, wherein said first **oligonucleotide**, when hybridized to said DNA analyte, is cleaved by said 5'-nuclease to (i) a first fragment that is substantially non-hybridizable to said DNA analyte and (ii) a second fragment that is 3' of said first fragment in said first **oligonucleotide** and is substantially hybridizable to said DNA analyte wherein at least a 100-fold molar excess, relative to said DNA analyte, of said first fragment and/or said second fragment is produced and (c) detecting the presence of said first fragment and/or said second fragment, the presence thereof indicating the presence of said DNA analyte.

18. The method of claim 17 wherein said first **oligonucleotide** has a substituent that facilitates separation of said first fragment or said second fragment from said medium.

19. The method of claim 17 wherein first fragment and/or said second fragment has a label.

20. The method of claim 19 wherein said label is selected from the group consisting of a member of a specific binding pair, dyes, fluorescent molecules, chemiluminescers, coenzymes, enzyme substrates, radioactive groups and suspendible particles.

21. The method of claim 17 wherein said second **oligonucleotide** hybridizes to said DNA analyte at a site contiguous with the site on said polynucleotide at which said first **oligonucleotide** hybridizes.

22. The method of claim 17 wherein said first **oligonucleotide** and/or said second **oligonucleotide** is DNA.

23. A method for detecting a polynucleotide analyte, said method comprising: (a) providing in combination a medium suspected of containing said polynucleotide analyte, a first DNA **oligonucleotide** at least a portion of which is capable of reversibly hybridizing with said polynucleotide analyte under isothermal conditions to form a complex, said isothermal conditions being at or near the melting temperature of said complex, a 5'-nuclease, a single nucleoside triphosphate and a second DNA **oligonucleotide** having the characteristic of hybridizing to a site on said polynucleotide analyte that is 3' of, and contiguous with, the site at which said first DNA **oligonucleotide** hybridizes wherein said polynucleotide analyte is substantially fully hybridized to said second DNA **oligonucleotide** under said isothermal conditions, (b) reversibly hybridizing under said isothermal conditions said polynucleotide analyte and said first DNA **oligonucleotide**, wherein said first DNA **oligonucleotide**, when hybridized to said polynucleotide analyte, is cleaved by said 5'-nuclease as a function of the presence of said polynucleotide analyte to provide, in at least a 100-fold molar excess of said polynucleotide analyte, (i) a first fragment that is substantially non-hybridizable to said polynucleotide analyte and (ii) a second fragment that is 3' of said first fragment in said first DNA **oligonucleotide** and is substantially hybridizable to said polynucleotide analyte, and (c) detecting the presence of said first fragment and/or said second fragment, the presence thereof indicating the presence of said polynucleotide analyte.

24. The method of claim 23 wherein said first fragment and/or said second fragment has a label.

25. The method of claim 24 wherein said label is selected from the group consisting of a member of a specific binding pair, dyes, fluorescent molecules, chemiluminescers, coenzymes, enzyme substrates, radioactive groups and suspendible particles.

26. The method of claim 23 wherein said polynucleotide analyte is DNA.

L5 ANSWER 103 OF 112 USPTAFULL on STN

97:112602 Nucleic acids specific for Rochalimaea quintana.

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Regnery, Russell L., Tucker, GA, United States

The United States of America as represented by the Department of Health and Human Services, Washington, DC, United States (U.S. government)

US 5693776 19971202

APPLICATION: US 1995-474499 19950607 (8)

DOCUMENT TYPE: Utility; Granted.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method of diagnosing cat scratch disease and a method of diagnosing bacillary angiomatosis in a subject by detecting the presence of Rochalimaea henselae or an immunogenically specific determinant thereof in the subject is provided. Also provided is a vaccine comprising an immunogenic amount of a nonpathogenic Rochalimaea henselae or an immunogenically specific determinant thereof and a pharmaceutically acceptable carrier. A method of diagnosing Rochalimaea quintana infection in a subject by detecting the presence of a nucleic acid specific to Rochalimaea quintana in a sample from the subject is provided. A purified heat shock protein of Rochalimaea is provided.

CLM What is claimed is:

1. An isolated nucleic acid consisting of the heat shock gene of Rochalimaea quintana, the nucleic acid having, at least about 18 nucleotides and not more than about 4000 nucleotides, a nucleotide fragment defined by a **primer** pair of SEQ ID NOS:1 and 2 and SEQ ID NOS:3 and 4, and at least about 18 nucleotides that selectively hybridizes with the nucleic acid defined in the Sequence Listing as SEQ ID NO:6.
2. The isolated nucleic acid of claim 1 in a vector suitable for expressing the nucleic acid.
3. The vector of claim 2 in a host suitable for expressing the nucleic acid.
4. An isolated nucleic acid specific for Rochalimaea quintana consisting of at least about 18 nucleotides and not more than about 4000 nucleotides, a nucleotide fragment defined by a **primer** pair of SEQ ID NOS:1 and 2 and SEQ ID NOS:3 and 4, and at least about 18 nucleotides that selectively hybridizes with the nucleic acid defined in the Sequence Listing as SEQ ID NO:6 under high stringency conditions of 62° C. for 1 h in 5×SSC, followed by two washes for 15 min each in 2×SSC containing 0.1% SDS at room temperature, followed by two washes of 15 min each at 52° C. in 0.5×SSC with 0.1% SDS.
5. The isolated nucleic acid of claim 4 in a vector suitable for expressing the nucleic acid.
6. The vector of claim 5 in a host suitable for expressing the nucleic acid.
7. An isolated Rochalimaea quintana-specific fragment of the heat shock gene of Rochalimaea quintana, the fragment of the heat shock gene consisting of a nucleotide fragment defined by a **primer** pair of SEQ ID NOS:1 and 2 and SEQ ID NOS:3 and 4, and at least about 18 nucleotides that selectively hybridizes with the nucleic acid defined in the Sequence Listing as SEQ ID NO:6.
8. The isolated nucleic acid of claim 7 in a vector suitable for expressing the nucleic acid.
9. The vector of claim 8 in a host suitable for expressing the nucleic acid.
10. The isolated nucleic acid of claim 1, wherein the fragment defined by the **primer** pair of SEQ ID NOS:1 and 2 and SEQ ID NOS:3 and 4 is at least about 414 base pairs.
11. The isolated nucleic acid of claim 4, wherein the fragment defined by the **primer** pair of SEQ ID NOS:1 and 2 and SEQ ID NOS:3 and 4 is at least about 414 base pairs.
12. The isolated Rochalimaea quintana-specific fragment of claim 7, wherein the fragment defined by the **primer** pair of SEQ ID NOS:1 and 2 and SEQ ID NOS:3 and 4 is at least about 414 base pairs.

L5 ANSWER 104 OF 112 USPTAFULL on STN

97:101637 Methods for producing a single stranded polydeoxynucleotide having two different defined sequences and kits.

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Ullman, Edwin F., Atherton, CA, United States
Hahnenberger, Karen M., Cupertino, CA, United States
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corporation)
US 5683879 19971104

APPLICATION: US 1995-475236 19950607 (8)

DOCUMENT TYPE: Utility; Granted.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method is disclosed for extending a primer to produce a single stranded polydeoxynucleotide that has two or more defined sequences. A combination is provided which comprises a template polynucleotide, a blocker polynucleotide, a primer polynucleotide and a polynucleotide Q. The template polynucleotide has three sequences T1, T2 and T3 wherein T1 is non-contiguous and 3' of T3 and wherein the 5' end of T3 is 5' of the 5' end of T2. The primer polynucleotide has a second defined sequence at its 3' end that is hybridizable with T1. The blocker polynucleotide has sequence B1 that is hybridizable with T3. Polynucleotide Q has sequences S1 and S2 wherein S1 is 3' of S2 and homologous with T2 and S2 is complementary to a first defined sequence that is to be introduced at the 3' end of the polynucleotide primer, when it is extended during the method of the invention. Polynucleotide Q is either attached to the 5' end of the blocker polynucleotide or present as a separate reagent. The primer is extended along the template polynucleotide and along at least a portion of sequence T2 and thereafter along the polynucleotide Q to give a single stranded polynucleotide having two or more defined sequences.

CLM What is claimed is:

1. A method for producing from a polynucleotide **primer** a single stranded polydeoxynucleotide having two different defined sequences P1 and P'2, said method comprising the steps of: (a) providing in combination (1) a template polynucleotide (template) having three sequences T1, T2 and T3 wherein T1 is non-contiguous with and 3' of said T2 and T3 and the 3' end of said T3 is contiguous with or lies within said T2, (2) a polynucleotide **primer** (**primer**) P1 whose 3' end is hybridizable with said T1, (3) a blocker polynucleotide with sequence B1, said B1 being hybridizable with said T3, and (4) polynucleotide Q having a sequence S1 and a sequence S2 wherein said Q is either attached at its 3'-end to the 5' end of said blocker polynucleotide or present as a separate reagent and wherein said S1 is 3' of said S2 and substantially identical to said T2 and wherein said S2 is complementary to said P'2, and (b) subjecting said combination to conditions for extending said **primer**, by means of a polymerase, P1 along the template and along at least a portion of said T2 and thereafter along said polynucleotide Q, wherein said polymerase has little or no 5'-3' exonuclease activity under said conditions for extending and wherein the 3'-end of said polynucleotide Q is not extended by said polymerase.

2. The method of claim 1 comprising the steps of dissociating said extended **primer** from it duplex, hybridizing a **primer** P2 to said P'2 and extending said **primer** along said extended **primer** to give a second extended **primer** characterized by a sequence P'1 that is 3' of said P2 and capable of hybridizing to said **primer** P1.

3. The method of claim 1 which comprises amplifying said extended **primers** by a **polymerase chain reaction**.

4. The method of claim 1 wherein said polynucleotide Q is attached to the 5' end of said blocker polynucleotide.

5. An isolated polynucleotide **primer** comprising a single strand of DNA comprised of a sequence T3 and a sequence T2 wherein the 3' end of said T3 is contiguous with the 5' end of T2, said single strand being complexed to a blocker DNA sequence comprised of a sequence B1 complementary to said T3 and a sequence S1 which is 5' of B1 and substantially identical to said T2.

6. The a **primer** polynucleotide of claim 5 having a sequence at its 3' end complexed with a sequence T1 in said single strand of DNA.

7. A kit comprising in packaged combination: (a) a **primer** polynucleotide having a sequence at its 3' end hybridizable with a first sequence T1 in a target polynucleotide (b) a blocker polynucleotide having a sequence B1, said B1 being hybridizable with a sequence T3 in said target polynucleotide wherein said T3 is 3' of said T1 and the 5' end of said T3 is 5' of the 5' end of a sequence T2, and (c) a polynucleotide Q having sequences S1 and S2 wherein S1 is 3' of said S2 and substantially identical to said T2 in said template polynucleotide and wherein said S2 is a sequence that is substantially identical to at least the 3' end portion of a second polynucleotide **primer**, the 3' end of said polynucleotide Q being incapable of chain extension in the presence of a polymerase.

blocker polynucleotide.

9. The kit of claim 7 wherein said polynucleotide Q is separate from said blocker polynucleotide.

10. The kit of claim 7 which comprises template dependent DNA polymerase.

11. The kit of claim 7 which comprises deoxynucleoside triphosphates.

12. The kit of claim 7 wherein one of said **primers** is labeled with a reporter molecule.

L5 ANSWER 105 OF 112 USPTAFULL on STN

97:96713 Method for introducing defined sequences at the 3' end of polynucleotides.

Laney, Maureen, Palo Alto, CA, United States

Chen, Yan, Palo Alto, CA, United States

Ullman, Edwin F., Atherton, CA, United States

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US 5679512 19971021

APPLICATION: US 1993-140349 19931020 (8)

DOCUMENT TYPE: Utility; Granted.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method is disclosed for extending a primer to produce a single stranded polydeoxynucleotide that has two or more defined sequences. A combination is provided which comprises a template polynucleotide, a blocker polynucleotide, a primer polynucleotide and a polynucleotide Q. The template polynucleotide has three sequences T1, T2 and T3 wherein T1 is non-contiguous and 3' of T3 and wherein the 5' end of T3 is 5' of the 5' end of T2. The primer polynucleotide has a second defined sequence at its 3' end that is hybridizable with T1. The blocker polynucleotide has sequence B1 that is hybridizable with T3. Polynucleotide Q has sequences S1 and S2 wherein S1 is 3' of S2 and homologous with T2 and S2 is complementary to a first defined sequence that is to be introduced at the 3' end of the polynucleotide primer, when it is extended during the method of the invention. Polynucleotide Q is either attached to the 5' end of the blocker polynucleotide or present as a separate reagent. The primer is extended along the template polynucleotide and along at least a portion of sequence T2 and thereafter along the polynucleotide Q to give a single stranded polynucleotide having two or more defined sequences.

CLM What is claimed is:

1. A method for producing a single stranded polynucleotide having two or more defined nucleic acid sequences, said method comprising the steps of: (a) hybridizing a sequence B1 of a blocker polynucleotide to a complementary sequence T3 within a template polynucleotide ("template"), said template being comprised of a sequence T1 and a sequence T2, wherein the 5' end of said T3 is 5' of the 5' end of said T2 and said T2 and T3 are 5' to and non-contiguous with said sequence T1, (b) hybridizing the 3' end of a polynucleotide **primer** comprising a second defined nucleic acid sequence to said T1 wherein step (b) is performed prior to, after, or simultaneously with step (a), and (c) extending said **primer**, by means of a polymerase, along said template and along at least a portion of said T2 and thereafter along a polynucleotide Q having sequences S1 and S2 wherein S1 is 3' of S2 and substantially identical with said T2 to give an extended **primer** having a first defined nucleic acid sequence at its 3' end that is complementary to S2, wherein polynucleotide Q is either attached to the 5' end of said blocker polynucleotide or is present as a separate reagent, wherein said polymerase has little or no 5'-3' exonuclease activity during said extending, and wherein the 3'-end of said polynucleotide Q, when present as a separate reagent, is not extended by said polymerase during said extending.

2. A method for producing from a **primer** polynucleotide a single stranded polydeoxynucleotide having two segments that are non-contiguous and complementary with each other, said method comprising the steps of: (a) providing in combination (a) a template polynucleotide having three sequences T1, T2 and T3 wherein T1 is non-contiguous and 3' of said T2 and T3 and the 3' end of said T3 is contiguous with or lies within said T2, (b) a **primer** polynucleotide whose 3' end is hybridizable with said T1 (c) a blocker polynucleotide with sequence B1, said B1 being hybridizable with said T3, and (d) a polynucleotide Q having sequence S1 and sequence S2 wherein said Q is either attached to the 5' end of said blocker polynucleotide or present as a separate reagent and wherein S1 is 3' of said S2 and substantially identical to said T2 and wherein said S2 is a sequence that is substantially identical to at least the 3' end of said **primer** polynucleotide, and (b) subjecting said combination to

said template polynucleotide and along at least a portion of said T2, and thereafter along said polynucleotide Q, wherein said polymerase has little or no 5'-3' exonuclease activity under said conditions for extending, and wherein the 3'-end of said polynucleotide Q, when present as a separate reagent, is not extended during said extending.

3. The method of claim 2 wherein at least a five base sequence within said B1 is comprised of at least 80% G and C nucleotides.

4. The method of claim 2 wherein said S1 is from 5 to 50 nucleotides in length.

5. The method of claim 2 wherein a sequence comprising the 5' end of said S1 is complementary to a sequence comprising the 5' end of said B1.

6. The method of claim 2 wherein the 5' end of said S1 is not complementary to the 5' end of said B1.

7. The method of claim 2 wherein said T2 is contiguous with said T3.

8. The method of claim 2 wherein the 3'-end of said polynucleotide Q is attached to the 5' end of said blocker polynucleotide.

9. A method of producing at least one copy of a target polynucleotide sequence, said method comprising the step of: providing in combination (1) a template polynucleotide having three sequences T1, T2 and T3 wherein T1 is non-contiguous and 3' of said T2 and T3 and wherein the 3' end of said T3 is contiguous with or lies within said T2 and wherein said target polynucleotide sequence is located between said T1 and T3, (2) a **primer** polynucleotide whose 3' end is hybridizable with said T1, (3) a blocker polynucleotide with sequence B1, said B1 being hybridizable with said T3, (4) a polynucleotide Q having sequences S1 and S2 wherein said Q is either attached to the 5' end of said blocker polynucleotide or present as a separate reagent and wherein S1 is 3' of S2 and substantially identical to said T2 and wherein said S2 is a sequence that is substantially identical to at least the 3' end of said **primer** polynucleotide, (5) DNA polymerase and (6) deoxynucleoside triphosphates under conditions wherein: (A) said blocker becomes hybridized to said template polynucleotide, (B) said **primer** becomes hybridized with and extended along said target polynucleotide sequence of said template polynucleotide and along at least a portion of said T2, and thereafter along said polynucleotide Q to form a duplex, (C) said extended **primer** is dissociated from the said duplex, and (D) said **primer** hybridizes with and is extended along said extended **primer** to form a duplex comprising extended **primer** containing a copy of said target sequence, wherein the 3'-end of said polynucleotide Q, when present as a separate reagent, is not extended by said DNA polymerase.

10. The method of claim 9 which comprises the step of examining for the presence of said extended **primer**.

11. The method of claim 9 wherein at least a five base sequence of said B1 is comprised of at least 80% G and C nucleotides.

12. The method of claim 9 wherein said S1 is from 5 to 50 nucleotides in length.

13. The method of claim 9 wherein a sequence comprising the 5' end of said S1 is complementary to a sequence comprising the 5' end of said B1.

14. The method of claim 9 wherein the 5' end of said S1 is not complementary to the 5' end of said B1.

15. The method of claim 9 wherein said T2 is contiguous with said T3.

16. The method of claim 9 wherein steps (C) and (D) are repeated.

17. The method of claim 9 wherein the 3'-end of said polynucleotide Q is attached to the 5' end of said blocker polynucleotide.

18. A method of forming multiple copies of a target polynucleotide sequence, said method comprising the steps of: (a) providing in combination (1) a template polynucleotide having three sequences T1, T2 and T3 wherein T1 is non-contiguous and 3' of said T2 and T3 and wherein the 3' end of said T3 is contiguous with or lies within said T2 and wherein said target polynucleotide sequence is located between said T1 and T3, (2) a **primer** polynucleotide whose 3' end is hybridizable with said T1, (3) a blocker polynucleotide with sequence B1, said B1 being hybridizable with said T3, (4) a polynucleotide Q having sequences S1 and S2 wherein said Q is either attached to the 5' end of said blocker polynucleotide or present as a separate reagent and wherein S1 is 3' of S2 and substantially identical to said T2 and wherein said S2 is a

primer polynucleotide, (5) DNA polymerase and (6) deoxynucleoside triphosphates under conditions wherein: (A) said blocker becomes hybridized to said template polynucleotide, (B) said **primer** becomes hybridized with and extended along said target polynucleotide sequence of said template polynucleotide and along at least a portion of said T2 and thereafter along said polynucleotide Q to form a duplex, (C) said extended **primer** is dissociated from the said duplex, and (D) said **primer** hybridizes with and is extended along said extended **primer** to form a duplex comprising extended **primer** containing a copy of said target sequence, wherein the 3'-end of said polynucleotide Q, when present as a separate reagent, is not extended by said DNA polymerase and (b) repeating steps (C) and (D).

19. The method of claim 18 which comprises examining for the presence of said extended **primer**.

20. The method of claim 18 wherein at least a five base sequence said B1 is comprised of at least 80% G and C nucleotides.

21. The method of claim 18 wherein said S1 is from 5 to 50 nucleotides in length.

22. The method of claim 18 wherein a sequence comprising the 5' end of said S1 is complementary to a sequence comprising the 5' end of said B1.

23. The method of claim 18 wherein the 5' end of S1 is not complementary to the 5' end of said B1.

24. The method of claim 18 wherein said T2 is contiguous with said T3.

25. The method of claim 18 wherein the 3'-end of said polynucleotide Q is attached to the 5' end of said blocker polynucleotide.

26. A method of forming multiple copies of a target polynucleotide sequence, said method comprising the steps of: (a) providing in combination (1) a template polynucleotide having three sequences T1, T2 and T3 wherein T1 is non-contiguous and 3' of said T2 and T3 and wherein the 5' end of said T3 is 5' of the 5' end of said T2 and wherein said target polynucleotide sequence is located between said T1 and T3, (2) a **primer** polynucleotide whose 3' end is hybridizable with said T1, (3) a blocker polynucleotide with sequence B1, said B1 being hybridizable with said T3, (4) a polynucleotide Q having sequences S1 and S2 wherein the 3'-end of said Q is attached to the 5' end of said blocker polynucleotide and wherein S1 is 3' of S2 and substantially identical to said T2 and wherein said S2 is a sequence that is substantially identical to at least the 3' end of said **primer** polynucleotide, (5) DNA polymerase and (6) deoxynucleoside triphosphates under conditions wherein: (A) said blocker becomes hybridized to said template polynucleotide, (B) said **primer** becomes hybridized with and extended along said target polynucleotide sequence of said template polynucleotide and along at least a portion of said T2 and thereafter along said polynucleotide Q to form a duplex, (C) said extended **primer** is dissociated from the said duplex, and (D) said **primer** hybridizes with and is extended along said extended **primer** to form a duplex comprising extended **primer** containing a copy of said target sequence, and (b) repeating steps (C) and (D).

27. The method of claim 26 which comprises examining for the presence of said extended **primer**.

28. The method of claim 26 wherein at least a five base sequence said B1 is comprised of at least 80% G and C nucleotides.

29. The method of claim 26 wherein said S1 is from 5 to 50 nucleotides in length.

30. The method of claim 26 wherein the 5' end of said S1 is complementary to the 5' end of said B1.

31. The method of claim 26 wherein the 5' end of said S1 is not complementary to the 5' end of said B1.

32. The method of claim 26 wherein said T2 is contiguous with said T3.

33. The method of claim 26 wherein said T2 is not contiguous with said T3.

34. A kit comprising in packaged combination: (a) a **primer** polynucleotide having a sequence at its 3' end hybridizable with a first sequence T1 in a target polynucleotide (b) a blocker polynucleotide having a sequence B1, said B1 being hybridizable with a sequence T3 in said target polynucleotide wherein said T3 is 3' of said T1 and the 5'

polynucleotide Q having sequences S1 and S2 wherein S1 is 3' of said S2 and substantially identical to said T2 in said template polynucleotide and wherein said S2 is a sequence that is substantially identical to at least the 3' end portion of said **primer**.

35. The kit of claim 34 wherein said polynucleotide Q is a part of said blocker polynucleotide.

36. The kit of claim 34 wherein said polynucleotide Q is separate from said blocker polynucleotide.

37. The kit of claim 34 which comprises template dependent DNA polymerase.

38. The kit of claim 34 which comprises deoxynucleoside triphosphates.

39. The kit of claim 34 wherein said **primer** is labeled with a reporter molecule.

L5 ANSWER 106 OF 112 USPATFULL on STN

97:22643 Method for producing a polynucleotide for use in single primer amplification.

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APPLICATION: US 1994-221662 19940401 (8)

DOCUMENT TYPE: Utility; Granted.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method is disclosed for extending an extender probe to produce a single stranded polydeoxynucleotide that is free of unreacted extender probe and has two segments that are non-contiguous and complementary with each other. The method comprises the steps of (1) providing in combination (a) a polynucleotide having two non-contiguous, non-complementary nucleotide sequences S1 and S2 wherein S2 is 5' of S1 and is at least ten deoxynucleotides long, (b) an extender probe comprised of two deoxynucleotide sequences, wherein the sequence at the 3'-end of the extender probe (EP1) is hybridizable with S1 and the other of the deoxynucleotide sequences (EP2) is substantially identical to S2 and (c) means for modifying the 3'-end of extender probe that does not hybridize with the polynucleotide and (2) extending the extender probe along the polynucleotide wherein extender probe not hybridized to the polynucleotide becomes modified at its 3'-end.

CLM What is claimed is:

1. A method for forming, from an extender probe, which maybe extended or modified, and a single stranded target polynucleotide sequence, a polynucleotide, said polynucleotide being complementary to an extended extender probe and having a sequence identical to said target polynucleotide sequence attached at its 3'-end to a polynucleotide sequence complementary to a polynucleotide sequence at the 5' end of said target polynucleotide sequence, wherein during said method said extender probe is modified to enhance the efficiency of said forming of said polynucleotide by reducing the priming effectiveness of said extender probe, said method comprising: (a) hybridizing to a sequence S1 at the 3'-end of said single stranded target polynucleotide sequence the 3'-end of said extender probe wherein said extender probe contains a sequence substantially identical to a sequence S2 at the 5'-end of said single stranded target polynucleotide sequence and wherein said S1 and said S2 are non-complementary and separated from one another by at least 10 nucleotides, (b) extending, by means of a polydeoxynucleotide polymerase and deoxynucleoside triphosphates, said extender probe along said single stranded target polynucleotide sequence to produce an extended extender probe, (c) extending or degrading by means of an enzyme said 3'-end of said extender probe not hybridized to said single stranded target polynucleotide sequence to produce a modified extender probe, (d) hybridizing a **primer** to the 3'-end of the extended extender probe, said **primer** having said sequence S2 at its 3'-end, and (e) extending, by means of a polydeoxynucleotide polymerase and deoxynucleoside triphosphates, said **primer** along said extended extender probe, thereby forming said polynucleotide having a sequence identical to said target polynucleotide sequence attached at its 3'-end to a polynucleotide sequence complementary to a polynucleotide sequence at the 5' end of said target polynucleotide sequence.

2. A method for producing from an extender probe a polydeoxynucleotide, said polydeoxynucleotide being an extended extender probe and having two segments that are non-contiguous and complementary with each other,

priming effectiveness thereof in an amplification of said polydeoxynucleotide, said method comprising: providing in combination (a) a polynucleotide having two non-contiguous, non-complementary nucleotide sequences S1 and S2 wherein S2 is 5' of S1 and is at least ten nucleotides long wherein said S1 and S2 are separated by at least 10 nucleotides, (b) an extender probe comprised of two deoxynucleotide sequences, wherein the sequence at the 3'-end of said extender probe, EP1, hybridizes with S1 and the other of said deoxynucleotide sequences, EP2, is homologous to S2, and (c) an enzyme for chemically modifying the 3'-end of said extender probe that does not hybridize with said polynucleotide, and extending, by means of a polydeoxynucleotide polymerase and polydeoxynucleoside triphosphates, said extender probe along said polynucleotide to produce extended extender probe which is said polydeoxynucleotide having two segments that are non-contiguous and complementary with each other, wherein the 3'-end of said extender probe not hybridized with said polynucleotide is extended or degraded by means of said enzyme, thereby producing modified extender probe.

3. The method of claim 2 wherein said enzyme is for extending the 3'-end of said extender probe.

4. The method of claim 3 wherein said combination further comprises a nucleotide sequence EP3 that hybridizes with said EP2.

5. The method of claim 3 wherein said combination further comprises a nucleotide sequence EP3 in said extender probe, wherein said extender probe not hybridized with the target polynucleotide sequence forms a loop and said EP3 hybridizes with said EP2.

6. The method of claim 3 wherein said EP3 is 3' of said EP2.

7. The method of claim 3 wherein said EP3 is 5' of said EP2.

8. The method of claim 2 wherein said enzyme is for degrading the 3'-end of said extender probe.

9. The method of claim 8 wherein said enzyme is an enzyme having 3'-exonuclease activity.

10. The method of claim 2 which further comprises replicating said polydeoxynucleotide by providing in said combination a polydeoxynucleotide **primer** that hybridizes at least at its 3'-end with a nucleotide sequence complementary to S2 under conditions where (1) said extended extender probe is rendered single stranded, (2) said polydeoxynucleotide **primer** hybridizes with and is extended along said extended extender probe to form a duplex comprising extended **primer**, (3) said extended **primer** is dissociated from said duplex, and (4) said polydeoxynucleotide **primer** hybridizes with and is extended along said extended **primer** to form a duplex comprising extended **primer**.

11. The method of claim 10 wherein said polydeoxynucleotide **primer** is comprised of sequence EP2 at its 3'-end and conditions are provided where (1) said extended extender probe is rendered single stranded, (2) said polydeoxynucleotide **primer** hybridizes with and is extended along said extended extender probe to form a duplex comprising extended **primer**, (3) said extended **primer** is dissociated from said duplex, and (4) said polydeoxynucleotide **primer** hybridizes with and is extended along said extended **primer** to form a duplex comprising extended **primer**.

12. The method of claim 10 wherein steps (3) and (4) are repeated.

13. The method of claim 12 wherein the concentration of said extender probe is substantially lower than that of said polydeoxynucleotide **primer**.

14. The method of claim 12 wherein the concentration of said extender probe is less than one percent that of said polydeoxynucleotide **primer**.

15. A method for replicating a target polynucleotide sequence, said target polynucleotide sequence having two non-contiguous, non-complementary nucleotide sequences S1 and S2 each at least 10 nucleotides long separated from one another by at least 10 nucleotides, wherein S2 is 5' of S1 and wherein during said method said extender probe is extended or degraded to reduce the priming effectiveness thereof in further replication of said target polynucleotide sequence, said method comprising: providing in combination, either concomitantly or wholly or partially sequentially, (1) said target polynucleotide sequence, (2) an extender probe, which may be extended or modified, having two deoxynucleotide sequences wherein the sequence at the 3'-end of said extender probe, EP1, hybridizes with S1 and the other of said deoxynucleotide sequences, EP2, is homologous to S2, (3) an enzyme for

with said target polynucleotide sequence, (4) a polydeoxynucleotide **primer** comprised of sequence S2 at its 3'-end where said polydeoxynucleotide **primer** may be provided directly or generated in situ, (5) DNA polymerase and (6) deoxynucleoside triphosphates under conditions wherein (A) some of said extender probe becomes hybridized with and extended along said target polynucleotide sequence to form a duplex comprising extended extender probe, (B) extender probe not hybridized to said target nucleotide sequence is extended or degraded at its 3'-end by said enzyme, (C) said extended extender probe is dissociated from said duplex, (D) said polydeoxynucleotide **primer** hybridizes with and is extended along said extended extender probe to form a duplex comprising extended polydeoxynucleotide **primer**, (E) said extended polydeoxynucleotide **primer** is dissociated from said duplex, and (F) said polydeoxynucleotide **primer** hybridizes with and is extended along said extended polydeoxynucleotide **primer** to form a duplex comprising extended polydeoxynucleotide **primer** and steps (E) and (F) are repeated, wherein said extended polydeoxynucleotide **primer** is a replication of said target polynucleotide sequence.

16. The method of claim 15 wherein said enzyme is for extending the 3'-end of said extender probe.

17. The method of claim 16 wherein said combination further comprises a nucleotide sequence EP3 that hybridizes with said EP2.

18. The method of claim 16 wherein said combination further comprises a nucleotide sequence EP3 in said extender probe, wherein said extender probe not hybridized with said target polynucleotide sequence forms a loop and said EP3 hybridizes with said EP2.

19. The method of claim 16 wherein said EP3 is 3' of said EP2.

20. The method of claim 16 wherein said EP3 is 5' of said EP2.

21. The method of claim 15 wherein said enzyme is for degrading the 3'-end of said extender probe.

22. The method of claim 21 wherein said enzyme is an enzyme having 3'-exonuclease activity.

23. The method of claim 21 wherein said combination further comprises a polynucleotide sequence NS3 that hybridizes with at least the 3'-end of said EP2.

24. The method of claim 23 wherein said polydeoxynucleotide **primer** is provided by degradation of said extender probe at least the 3'-end of which is not hybridized with the polynucleotide sequence NS3.

25. The method of claim 23 wherein said NS3 is part of said extender probe.

26. The method of claim 15 wherein steps (E) and (F) are repeated at least three times.

27. The method of claim 15 wherein the concentration of said extender probe is less than one percent that of said polydeoxynucleotide **primer**.

28. The method of claim 15 wherein at least a fifteen nucleotide sequence of said extender probe hybridizes with S1.

29. The method of claim 15 wherein said polydeoxynucleotide **primer** contains at least a fifteen nucleotide sequence identical to S2.

30. The method of claim 15 wherein S1 and S2 each respectively contain from 10 to 100 nucleotides.

31. The method of claim 15 wherein said target polynucleotide sequence is DNA.

32. The method of claim 15 wherein said polydeoxynucleotide **primer** contains a nucleotide sequence in addition to S2.

33. A kit comprising in packaged combination: an extender probe having at its 3'-end a sequence (EP1) that hybridizes with a first sequence in a target polynucleotide sequence and having a sequence (EP2) that is substantially identical to a second sequence of said target polynucleotide sequence, wherein in said target polynucleotide sequence said second sequence is 5' and non-contiguous with said first sequence, a nucleotide sequence (NS) having a portion that hybridizes with EP1 wherein said NS may be a separate molecule or part of said extender probe, and a polydeoxynucleotide **primer** that hybridizes with a sequence that is complementary with said second sequence.

34. The kit of claim 33 wherein said NS is part of said extender probe.
35. The kit of claim 33 wherein said NS is a molecule separate from said extender probe.
36. The kit of claim 33 which comprises template dependent DNA polymerase.
37. The kit of claim 36 which comprises deoxynucleoside triphosphates.
38. The kit of claim 33 wherein said polydeoxynucleotide **primer** is labeled with a reporter molecule.
39. A kit comprising in packaged combination: an extender probe having at its 3'-end a sequence that hybridizes with a first sequence (EP1) in a target polynucleotide sequence and having a sequence that is substantially identical to a second sequence (EP2) of said target polynucleotide sequence, wherein in said target polynucleotide sequence said second sequence is 5' and non-contiguous with said first sequence, an enzyme that degrades said sequence that hybridizes with said EP1 when said sequence is not hybridized to said EP1, and a polydeoxynucleotide **primer** that hybridizes with a sequence that is complementary with said second sequence.
40. The kit of claim 39 wherein said enzyme has 3' exonuclease activity.
41. The kit of claim 39 which comprises a nucleotide sequence (NS) that hybridizes with at least a portion of said EP2 such that, upon degradation of excess of said extender probe, said polydeoxynucleotide **primer** is formed in situ.
42. The kit of claim 41 wherein said NS is part of said extender probe.
43. The kit of claim 41 wherein said NS is a molecule separate from said extender probe.
44. The kit of claim 41 which comprises template dependent DNA polymerase.
45. The kit of claim 44 which comprises deoxynucleoside triphosphates.
46. The kit of claim 39 wherein said polydeoxynucleotide **primer** is labeled with a reporter molecule.

L5 ANSWER 107 OF 112 USPATFULL on STN

97:5872 Method for producing a polynucleotide for use in single primer amplification.

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US 5595891 19970121

APPLICATION: US 1990-555323 19900719 (7)

DOCUMENT TYPE: Utility; Granted.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method is disclosed for producing a single stranded polydeoxynucleotide having two segments that are non-contiguous and complementary with each other. The method comprises the steps of providing in combination (1) a polynucleotide having two non-contiguous, non-complementary nucleotide sequences S1 and S2 wherein S2 is 5' of S1 and is at least ten deoxynucleotides long and (2) an extender probe comprised of two deoxynucleotide sequences, wherein the sequence at the 3'-end of the extender probe is hybridizable with S1 and the other of the deoxynucleotide sequences is homologous to S2 and (b) extending the extender probe along the polynucleotide. The method can also comprise providing in the combination a polydeoxynucleotide primer capable of hybridizing at least at its 3'-end with a nucleotide sequence complementary to S2 under conditions where (1) the extended extender probe is rendered single stranded, (2) the polydeoxynucleotide primer hybridizes with and is extended along the extended extender probe to form a duplex comprising extended primer, (3) the extended primer is dissociated from the duplex, and (4) the primer hybridizes with and is extended along the extended primer to form a duplex comprising extended primer, and repeating steps (3) and (4). The method finds particular application in the detection of polynucleotide analytes.

CLM What is claimed is:

1. A method for producing a single stranded polydeoxynucleotide having two segments that are non-contiguous and complementary with each other, said method comprising the steps of: providing in combination a

sequences S1 and S2 wherein S2 is 5' of S1 and is at least ten nucleotides long and an extender probe comprised of two deoxynucleotide sequences, wherein the sequence at the 3' end of said extender probe is hybridizable with S1 and the other of said deoxynucleotide sequences is homologous to S2 DNA polymerase and deoxynucleoside triphosphates, and extending said extender probe along said polynucleotide, thereby producing said single stranded polydeoxynucleotide.

2. The method of claim 1 which further comprises providing in said combination a polydeoxynucleotide **primer** capable of hybridizing at least at its 3'-end with a nucleotide sequence complementary to S2 under conditions where (a) said extended extender probe is rendered single stranded, (b) said polydeoxynucleotide **primer** hybridizes with and is extended along said extended extender probe to form a duplex comprising extended **primer**, (c) said extended **primer** is dissociated from said duplex, and (d) said **primer** hybridizes with and is extended along said extended **primer** to form a duplex comprising extended **primer**.

3. The method of claim 2 wherein steps (c) and (d) are repeated.

4. The method of claim 3 wherein the concentration of said extender probe is substantially lower than that of said polydeoxynucleotide **primer**.

5. The method of claim 3 wherein the concentration of said extender probe is less than one percent that of said polydeoxynucleotide **primer**.

6. A method for producing multiple copies of a single stranded polydeoxynucleotide having two segments that are non-contiguous and complementary with each other, said method comprising the step of: providing in combination, either concomitantly or wholly or partially sequentially, a polynucleotide having two non-contiguous, non-complementary nucleotide sequences, S1 and S2, wherein S2 is 5' of S1 and is at least ten nucleotides long, an extender probe comprised of two deoxynucleotide sequences, wherein the sequence at the 3' end of said extender probe is hybridizable with S1 and the other of said nucleotide sequences is homologous to S2 and not complementary to said polynucleotide, a polydeoxynucleotide **primer** capable of hybridizing at least at its 3' end with a nucleotide sequence complementary to S2, DNA polymerase, and deoxynucleoside triphosphates under conditions where (a) said extender probe is extended along said polynucleotide to form a duplex, (b) said extended extender probe is dissociated from said duplex, (c) said polydeoxynucleotide **primer** hybridizes with and is extended along said extended extender probe to form a second duplex comprising extended **primer** (d) said extended **primer** is dissociated from said second duplex, and (e) said **primer** hybridizes with and is extended along said extended **primer** to form a duplex comprising extended **primer**, and steps (d) and (e) are repeated, thereby producing multiple copies of said single stranded polynucleotide.

7. The method of claim 6 wherein steps (d) and (e) are repeated at least three times.

8. The method of claim 6 wherein the concentration of said extender probe is less than one percent that of said polydeoxynucleotide **primer**.

9. The method of claim 6 wherein at least a fifteen deoxynucleotide sequence of said extender probe hybridizes with S1.

10. The method of claim 6 wherein said polydeoxynucleotide **primer** contains at least a fifteen deoxynucleotide sequence capable of hybridizing with a sequence complementary to S2.

11. The method of claim 6 wherein said polynucleotide is DNA.

12. A method for detecting the presence of a target nucleotide sequence in a medium suspected of containing said target nucleotide sequence, said target nucleotide sequence having two non-contiguous, non-complementary nucleotide sequences S1 and S2 wherein S2 is 5' of S1 and at least 10 nucleotides long, said method comprising the steps of: (a) providing in combination, either concomitantly or wholly or partially sequentially, said medium, an extender probe having two deoxynucleotide sequences wherein the sequence at the 3' end of said extender probe is hybridizable with S1 and the other of said deoxynucleotide sequences is homologous to S2 and not complementary to said target nucleotide sequence, a polydeoxynucleotide **primer** capable of hybridizing with a nucleotide sequence complementary to S2, DNA polymerase and deoxynucleoside triphosphates under conditions wherein (i) said extender probe is extended along said polynucleotide to form a duplex, (ii) said extended extender probe is dissociated from said duplex, (iii) said **primer** hybridizes with and is extended along said extended extender probe to form a duplex comprising extended **primer**,

said **primer** hybridizes with and is extended along said extended **primer** to form a duplex comprising extended **primer** and steps (iv) and (v) are repeated, and (b) examining for the presence of said extended **primer** to detect the presence of said target nucleotide sequence.

13. The method of claim 12 wherein steps (iv) and (v) are repeated at least three times.

14. The method of claim 12 wherein the concentration of said extender probe is less than one percent that of said polydeoxynucleotide **primer**.

15. The method of claim 12 wherein at least a fifteen nucleotide sequence of said extender probe hybridizes with S1.

16. The method of claim 12 wherein said polydeoxynucleotide **primer** contains at least a fifteen nucleotide sequence capable of hybridizing with a sequence complementary to S2.

17. The method of claim 12 wherein S1 and S2 each respectively contain from 10 to 100 nucleotides.

18. The method of claim 12 wherein said target nucleotide sequence is DNA.

19. The method of claim 12 wherein the concentration of said extender probe is less than 1 nM and the concentration of said polydeoxynucleotide **primer** is greater than 100 nM.

20. The method of claim 12 wherein said polydeoxynucleotide **primer** is labeled with a reporter molecule.

21. The method of claim 12 wherein said polydeoxynucleotide **primer** contains a nucleotide sequence other than the sequence that hybridizes with said sequence complementary to S2.

22. The method of claim 21 wherein said presence of said extended **primer** is detected by examining for a reporter molecule covalently bonded to a nucleotide sequence that is complementary to a portion of said target nucleotide sequence other than S1 or S2.

23. A method for detecting the presence of a polynucleotide analyte in a sample suspected of containing said polynucleotide analyte, said method comprising the steps of: (a) treating a medium containing said sample to form a single stranded target nucleotide sequence from said polynucleotide analyte, if present, said target nucleotide sequence having two non-contiguous, non-complementary nucleotide sequences S1 and S2 wherein S2 is 5' of S1, and is at least ten nucleotides long, (b) combining said medium with an extender probe having two deoxynucleotide sequences wherein the sequence at the 3' end of said extender probe is hybridizable with S1 and the other of said deoxynucleotide sequences is homologous to S2 and not complementary to said target sequence, a polydeoxynucleotide **primer** capable of hybridizing with a nucleotide sequence complementary to S2, deoxynucleoside triphosphates, and DNA template dependent polydeoxynucleotide polymerase under conditions wherein (i) said extender probe is hybridized with and is extended along said target nucleotide sequence to form a duplex, (ii) said extended extender probe is dissociated from said duplex, (iii) said **primer** hybridizes with and is extended along said extended extender probe to form a duplex comprising extended **primer**, (iv) said extended **primer** is dissociated from said duplex, and (v) said **primer** hybridizes with and is extended along said extended **primer** to form a duplex comprising extended **primer** and steps (iv) and (v) are repeated, wherein steps (a) and (b) are performed concomitantly or wholly or partially sequentially, and (c) examining for the presence of said extended **primer** to detect the presence of said polynucleotide analyte.

24. The method of claim 23 wherein steps (iv) and (v) are repeated less than 30 times.

25. The method of claim 23 wherein the concentration of said extender probe is less than one percent that of said polydeoxynucleotide **primer**.

26. The method of claim 23 wherein at least a fifteen nucleotide sequence of said extender probe hybridizes with S1.

27. The method of claim 23 wherein said polydeoxynucleotide **primer** contains at least a fifteen nucleotide sequence capable of hybridizing with a sequence complementary to S2.

28. The method of claim 23 wherein S1 and S2 each respectively contain from 10 to 100 nucleotides.

29. The method of claim 23 wherein said polynucleotide analyte is DNA.

30. The method of claim 23 wherein said polynucleotide analyte is RNA and said medium includes reverse transcriptase.

31. The method of claim 23 wherein the concentration of said extender probe is less than 1 nM and the concentration of said polydeoxynucleotide **primer** is greater than 100 nM.

32. The method of claim 23 wherein said polydeoxynucleotide **primer** is labeled with a reporter molecule.

33. The method of claim 32 wherein step (c) includes examining for the presence of said reporter molecule covalently bonded to a nucleotide sequence complementary to a sequence present in said target nucleotide sequence other than S1 and S2.

34. The method of claim 23 wherein said deoxynucleoside triphosphates are dATP, dGTP, dCTP, and dTTP.

35. The method of claim 23 wherein steps (4) and 5) are repeated such that the number of said duplexes formed is increased by at least a factor of 1000.

36. The method of claim 32 wherein said reporter molecule is selected from the group consisting of fluorescers, chemilumescers, promoters, co-enzymes, radioactive substances, amplifiable polynucleotide sequences, small organic molecules, catalysts and polynucleotide sequences coding for catalysts.

37. The method of claim 23 wherein said polydeoxynucleotide **primer** is labeled with a ligand.

38. The method of claim 23 wherein said polydeoxynucleotide **primer** contains a nucleotide sequence other than the sequence that hybridizes with said sequence complementary to S2.

39. The method of claim 38 wherein said nucleotide sequence of said polydeoxynucleotide **primer** contains a sequence that, when hybridized to its complementary sequence, can be bound specifically by a receptor.

40. The method of claim 39 wherein said receptor is selected from the group consisting of repressors, activators, and nucleases.

41. The method of claim 38 wherein said nucleotide sequence of said polydeoxynucleotide **primer** contains a sequence that when hybridized to its complementary sequence, can be bound specifically by a receptor, and said extended **primer** is detected by binding said receptor to said extended **primer**.

42. A kit comprising in packaged combination: an extender probe having at its 3' end a sequence hybridizable with a first sequence in a target nucleotide sequence and having a sequence that is homologous to a second sequence of said target nucleotide sequence, wherein in said target nucleotide sequence said second sequence is 5' and non-contiguous with said first sequence, a polydeoxynucleotide **primer** capable of hybridizing with a sequence that is complementary with said second sequence, template dependent DNA polymerase, and deoxynucleoside triphosphates.

43. The kit of claim 42 wherein the amount of said extender probe is less than one percent the amount of said polydeoxynucleotide **primer**.

44. The kit of claim 42 wherein said extender probe comprises a 10 to 100 nucleotide sequence hybridizable with a target nucleotide sequence.

45. The kit of claim 42 wherein said extender probe comprises a 10 to 100 nucleotide sequence that is homologous to a sequence in a target nucleotide sequence.

46. The kit of claim 42 wherein said polydeoxynucleotide **primer** comprises a 10 to 100 nucleotide sequence capable of hybridizing with a sequence that is complementary to said second sequence.

47. The kit of claim 42 wherein said polydeoxynucleotide **primer** is labeled with a reporter molecule.

48. The kit of claim 42 wherein said polydeoxynucleotide **primer** is labeled with a ligand.

49. The kit of claim 42 wherein said polydeoxynucleotide **primer** comprises a nucleotide sequence in addition to that capable of

L5 ANSWER 108 OF 112 USPATFULL on STN

96:31728 Nucleic acid amplification using single primer.

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US 5508178 19960416

APPLICATION: US 1994-194140 19940209 (8)

DOCUMENT TYPE: Utility; Granted.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method is disclosed for determining the presence of a polynucleotide analyte in a sample suspected of containing the analyte. The method comprises (a) forming as a result of the presence of an analyte a single stranded polynucleotide comprising a target polynucleotide binding sequence flanked by first and second polynucleotide sequences that differ from the sequence of the analyte or a sequence complementary to the analyte sequence, (b) forming multiple copies of the single stranded polynucleotide, and (c) detecting the single stranded polynucleotide. Also disclosed is a method of producing at least one copy of a single stranded polynucleotide. The method comprises (a) forming in the presence of nucleoside triphosphates and template dependent polynucleotide polymerase an extension of a polynucleotide primer at least the 3'-end of which has at least a 10 base sequence hybridizable with a second sequence flanking the 3'-end of the single stranded polynucleotide, the second sequence being partially or fully complementary with at least a 10 base first sequence flanking the 5' end of the single stranded polynucleotide, (b) dissociating the extended polynucleotide primer and the single stranded polynucleotide, (c) repeating step a and (d) dissociating the extended polynucleotide primer and the copy of the single stranded polynucleotide.

CLM What is claimed is:

1. A method of producing at least one copy of a single stranded polynucleotide which comprises: (a) forming in the presence of nucleoside triphosphates and template dependent polynucleotide polymerase along a single stranded polynucleotide, flanked at the 5'-end and the 3'-end, respectively, by a first flanking sequence and a second flanking sequence, an extension of a polynucleotide **primer** at least the 3'-end of said polynucleotide **primer** having at least a 10 base sequence hybridizable with a 10 base sequence of said second flanking sequence, said 10 base sequence of said second flanking sequence being at least partially complementary, and thus hybridizable, with said first flanking sequence; (b) dissociating said extended polynucleotide **primer** and said single stranded polynucleotide; and (c) repeating step a wherein said polynucleotide **primer** hybridizes to and is extended along (1) said single stranded polynucleotide and (2) said extension of said polynucleotide **primer**.

2. The method of claim 1 wherein the last 10 nucleotides of the 3'-end of said polynucleotide **primer** are complementary to said second flanking sequence.

3. The method of claim 1 wherein said first flanking sequence is fully complementary to said second flanking sequence.

4. The method of claim 1 which further comprises the step of dissociating said extended polynucleotide **primer** and said copy.

5. The method of claim 1 wherein said single stranded polynucleotide or said copy is DNA.

6. The method of claim 5 wherein the product formed after repeating step (a) contains an inverted repeat.

7. The method of claim 1 wherein said hybridizable sequence of said polynucleotide **primer** is 15 to 100 nucleotides in length.

8. The method of claim 1 wherein said template-dependent polynucleotide polymerase is a DNA polymerase and said nucleoside triphosphates are dATP, dGTP, dCTP, and dTTP.

9. The method of claim 1 wherein said method is carried out at a substantially excess concentration of said polynucleotide **primer** relative to the concentration of said single stranded polynucleotide.

number of said copies of said single stranded polynucleotide is increased by at least a factor of a thousand.

11. A method of producing multiple copies of a polynucleotide sequence, which comprises: (a) providing in combination (1) a single stranded polynucleotide having said polynucleotide sequence and being flanked at each end by at least partially complementary first and second flanking sequences, (2) a polynucleotide **primer** at least a 10 base portion of which at its 3'-end is hybridizable to a 10 base sequence of that member of said first and second flanking sequences that is at the 3'-end of said single stranded polynucleotide, said 10 base sequence being hybridizable to a 10 base sequence of that member of said first and second flanking sequences that is at the 5'-end of said single stranded polynucleotide, (3) nucleoside triphosphates, and (4) template dependent polynucleotide polymerase and (b) incubating said combination under conditions for either wholly or partially sequentially or concomitantly (1) dissociating said single stranded polynucleotide from any complementary sequences, (2) hybridizing said polynucleotide **primer** with the flanking sequence at the 3' end of said single stranded polynucleotide, (3) extending said polynucleotide **primer** along said single stranded polynucleotide to provide a first extended polynucleotide **primer**, (4) dissociating said first extended polynucleotide **primer** and said single stranded polynucleotide, (5) hybridizing said first extended polynucleotide **primer** with said polynucleotide **primer**, (6) extending said polynucleotide **primer** along said first extended polynucleotide **primer** to provide a second extended polynucleotide **primer**, (7) dissociating said second extended polynucleotide **primer** from said first extended polynucleotide **primer**, and (8) repeating steps (5)-(7) above.

12. The method of claim 11 wherein said first and second sequences are fully complementary.

13. The method of claim 11 wherein said single stranded polynucleotide sequence or said first or second extended polynucleotide **primer** is DNA.

14. The method of claim 11 wherein said first extended polynucleotide **primer** when hybridized with said second extended polynucleotide **primer** contains an inverted repeat.

15. The method of claim 11 wherein said polynucleotide **primer** is 15 to 100 nucleotides in length.

16. The method of claim 11 wherein said template-dependent polynucleotide polymerase is a DNA polymerase and said nucleoside triphosphates are dATP, dGTP, dCTP, and dTTP.

17. The method of claim 11 wherein said method is carried out at a substantially excess concentration of said polynucleotide **primer** relative to the concentration of said single stranded polynucleotide.

18. The method of claim 11 wherein the number of said copies of said polynucleotide sequence is increased by at least a factor of a thousand.

19. A method of producing multiple copies of a polynucleotide sequence in a polynucleotide, said sequence being flanked at each end by a different member of a pair of flanking sequences that are at least partially complementary to each other, which comprises: (a) combining said polynucleotide with a single polynucleotide **primer** having at least a terminal sequence at its 3' end at least partially complementary to and hybridizable with at least a portion of the member of said pair of flanking sequences at the 3'-end of said polynucleotide sequence, said portion being hybridizable with the member of said pair of flanking sequences at the 5'-end of said polynucleotide sequence, nucleoside triphosphates, and template dependent polynucleotide polymerase, (b) incubating said combination under conditions for either wholly or partially sequentially or concomitantly (1) dissociating said polynucleotide sequence from any sequence with which it is hybridized to provide a single stranded polynucleotide, (2) hybridizing said polynucleotide **primer** with the flanking sequence at the 3'-end of said single stranded polynucleotide, (3) extending said polynucleotide **primer** along said single stranded polynucleotide to provide an extended polynucleotide **primer**, (4) dissociating said first extended polynucleotide **primer** and said single stranded polynucleotide, (5) hybridizing said first extended polynucleotide **primer** with said polynucleotide **primer**, (6) extending said polynucleotide **primer** along said first extended polynucleotide **primer** to provide a second extended polynucleotide **primer**, (7) dissociating said second extended polynucleotide **primer** from said first extended polynucleotide **primer**, and (8) repeating steps (5)-(7) above.

20. The method of claim 19 wherein said polynucleotide is DNA.

21. The method of claim 19 wherein said polynucleotide **primer** is 15 to 100 nucleotides in length.

22. The method of claim 19 wherein said template-dependent polynucleotide polymerase is a DNA polymerase and said nucleoside triphosphates are dATP, dGTP, dCTP, and dTTP.

23. The method of claim 19 wherein said method is carried out at a substantially excess concentration of said polynucleotide **primer** relative to the concentration of said polynucleotide.

24. The method of claim 19 wherein the concentration of reagents and at least one temperature are chosen to substantially optimize binding of said polynucleotide **primer** to said single stranded polynucleotide.

25. The method of claim 19 which further comprises the step of detecting at least one of said extended **primers**.

L5 ANSWER 109 OF 112 USPATFULL on STN

95:71247 Method for producing a polynucleotide having an intramolecularly base-paired structure.

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US 5439793 19950808

APPLICATION: US 1990-555968 19900719 (7)

DOCUMENT TYPE: Utility; Granted.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method is disclosed for forming a single stranded polynucleotide having two segments that are non-contiguous and hybridizable with each other. The method comprises the step of providing in combination (1) a first polynucleotide sequence having a hydroxyl at its 3'-end, (2) a second polynucleotide sequence having a hydroxyl or phosphate group at its 5'-end, and (3) a ligase, wherein at least ten consecutive bases of one of the sequences can hybridize to the other of the sequences to form a duplex. The duplex is comprised of a non-hybridized single stranded portion of one of the polynucleotide sequences containing one of the ends and at least five bases. The combination is provided under conditions for forming the duplex and ligating the ends within the duplex. The method finds particular application in the detection of polynucleotide analytes.

CLM What is claimed is:

1. A method of forming a single stranded polynucleotide having two segments that are non-contiguous and hybridizable with each other, said method comprising the steps of: (a) forming a partially single stranded duplex by hybridizing (1) a first polynucleotide sequence of about 30 to 5000 nucleotides in length and having one of a hydroxyl or phosphate group at its 3'-end with (2) a second polynucleotide sequence of at least 10 consecutive nucleotides in length that hybridizes with said first polynucleotide sequence and having the other of a hydroxyl or phosphate group at its 5'-end, wherein said partially single stranded duplex is comprised of a non-hybridized single stranded portion of at least one of said first and second polynucleotide sequences containing one of said ends and 10 to 25 nucleotides in length, and (b) ligating said ends within said duplex with an excess of T4 deoxyribonucleic acid ligase, thereby forming a single stranded polynucleotide having two segments that are non-contiguous and hybridizable with each other.

2. The method of claim 1 wherein said second polynucleotide sequence has a phosphate group at its 5'-end.

3. The method of claim 1 wherein said 5'-end of said second sequence is hybridized in said duplex.

4. The method of claim 3 wherein said 5'-end has a phosphate group.

5. The method of claim 1 wherein said partially single stranded duplex is comprised of a non-hybridized single stranded portion of each of said first and second polynucleotide sequences single stranded portion, each containing one of said ends and 5 to 25 nucleotides, wherein said 5'-end has a phosphate group.

6. A method of forming a single stranded polynucleotide having two segments that are non-contiguous and hybridizable with each other, said method comprising the steps of: (a) combining (1) a first polynucleotide sequence having about 30 to 5000 nucleotides, (2) a second polynucleotide sequence having at least 10 consecutive nucleotides that can hybridize with said first polynucleotide sequence, and (3) a T4 deoxyribonucleic acid ligase wherein said ligase is present in a concentration in excess relative to the concentration of said first and second polynucleotide sequences; (b) hybridizing said first and second

sequences (sequence A) and the 5'-end of the other sequence (sequence B) is bound to the corresponding end of the hybridized first and second polynucleotide sequences by a single stranded sequence of 10 to 25 nucleotides in length; and (c) forming a phosphodiester to and between the 5'-end of sequence B and the 3'-end of sequence A thereby forming a single stranded polynucleotide having two segments that are noncontiguous and hybridizable with each other.

7. The method of claim 6 wherein the 5'-end of sequence B has a phosphate group.

8. The method of claim 7 wherein the nucleotide at the 5'-end of sequence B is cytidine.

9. The method of claim 6 wherein the 3'-end of sequence A or the 5'-end of sequence B is part of said hybridized first and second polynucleotide sequences segment.

10. The method of claim 6 wherein said noncontiguous hybridizable segments contain from 10 to 100 nucleotides.

11. The method of claim 6 wherein said noncontiguous hybridized segments contain at least 10 nucleotides complementary to each other.

12. The method of claim 6 wherein said single stranded polynucleotide having two segments that are noncontiguous and hybridizable with each other is deoxyribonucleic acid (DNA).

13. The method of claim 6 wherein said ligase is present in a concentration of about 500 to 100 fold excess relative to the concentration of said first and second sequences.

14. A method for detecting the presence of a target polynucleotide sequence in a medium suspected of containing said target polynucleotide sequence, said method comprising the steps of: (a) combining said medium with (1) a polynucleotide wherein said polynucleotide and said target polynucleotide sequence each have a segment of at least 10 nucleotides hybridizable with each other, the 5'-end of said polynucleotide or said target polynucleotide sequence having a phosphate group and (2) a T4 deoxyribonucleic acid ligase wherein said ligase is present in a concentration in excess relative to the concentrations of said polynucleotide and said target polynucleotide sequence, (b) hybridizing said polynucleotide and said target polynucleotide sequence, if present, to form a partially single stranded duplex comprised of at least one non-hybridized single stranded portion 10 to 25 nucleotides in length at one end of one of said polynucleotide and said target polynucleotide sequence wherein a first terminal nucleotide of said non-hybridized single-stranded portion and a second terminal nucleotide of said other member of said duplex are ligated by said T4 deoxyribonucleic acid ligase, said second terminal nucleotide being proximal said first terminal nucleotide in said duplex, (c) forming multiple copies of said target polynucleotide sequence ligated to said polynucleotide and detecting said copies, thereby detecting the presence of said target polynucleotide sequence.

15. The method of claim 14 wherein said multiple copies are formed by: (a) hybridizing a single stranded polynucleotide **primer** at its 3'-end to a sequence within said segment of either said target polynucleotide sequence or said polynucleotide, both as part of said partially single stranded duplex comprised of said target polynucleotide sequence ligated to said polynucleotide, (b) extending said polynucleotide **primer** in the presence of nucleoside triphosphates and a polynucleotide polymerase to provide a first extended polynucleotide **primer**, (c) dissociating said first extended polynucleotide **primer** from said sequence within said hybridizable sequence, (d) hybridizing said first extended polynucleotide **primer** with said polynucleotide **primer**, (e) extending said polynucleotide **primer** along said first extended polynucleotide **primer** to provide a second extended polynucleotide **primer**, (f) dissociating said second extended polynucleotide **primer** from said first extended polynucleotide **primer**, and (g) repeating steps (d)-(f) above.

16. The method of claim 15 wherein said polynucleotide **primer** is 10 to 100 nucleotides in length.

17. The method of claim 14 wherein said target polynucleotide sequence is deoxyribonucleic acid (DNA).

18. The method of claim 14 wherein said first terminal nucleotide has a 5'-phosphate group and said second terminal nucleotide has a 3'-hydroxyl group.

polynucleotide sequence is separated from said hybridizable segment by 10 to 25 nucleotides.

20. The method of claim 18 wherein said first terminal nucleotide is cytidine (C).

21. The method of claim 14 wherein each of said segments has a sequence of from 10 to 100 nucleotides hybridizable with the other.

22. The method of claim 14 wherein each of said segments has at least a 10 nucleotide sequence complementary to the other.

23. The method of claim 14 wherein said polynucleotide is deoxyribonucleic acid (DNA).

24. The method of claim 14 wherein said ligase is present in a concentration of about 500 to 100 fold excess relative to the concentration of said polynucleotide and said target nucleotide sequences.

25. A method for detecting the presence of a polynucleotide analyte in a sample suspected of containing said polynucleotide analyte, said method comprising the steps of: (a) combining said sample with (1) a polynucleotide having at least a 10 nucleotide segment hybridizable with a segment of said polynucleotide analyte, and (2) a T4 deoxyribonucleic acid ligase in a concentration in excess relative to the concentrations of said polynucleotide and said polynucleotide analyte, (b) hybridizing said polynucleotide and said polynucleotide analyte, if present, to form a partially single-stranded duplex comprised of at least one nonhybridized single stranded portion 10 to 25 nucleotides in length at one end of said partially single stranded duplex wherein a first terminal nucleotide of said nonhybridized single-stranded portion and a second terminal nucleotide of the other member of said duplex are ligated by said T4 deoxyribonucleic acid ligase, said second terminal nucleotide being proximal said first terminal nucleotide in said duplex, (c) combining the sample suspected of containing said polynucleotide analyte ligated to said polynucleotide in said partially single-stranded duplex, nucleoside triphosphates and template-dependent polynucleotide polymerase and a polynucleotide **primer** at least the 3'-end of which hybridizes within said duplex with a sequence of either said polynucleotide analyte or of said polynucleotide, (d) forming an extended polynucleotide **primer**, and (e) examining said sample for the presence of extended polynucleotide **primer** thereby detecting the presence of said polynucleotide analyte.

26. The method of claim 25 wherein a portion of said polynucleotide **primer** is labeled with a first reporter group and a portion is labeled with a second reporter group.

27. The method of claim 25 wherein said polynucleotide analyte is deoxyribonucleic acid (DNA).

28. The method of claim 25 wherein said polynucleotide **primer** is 20 to 100 nucleotides in length.

29. The method of claim 25 wherein said template-dependent polynucleotide polymerase is a deoxyribonucleic acid (DNA) polymerase and said nucleotide triphosphates are deoxyadenosine triphosphate (ATP), deoxyguanosine triphosphate (dGTP), deoxycytidine triphosphate (dCTP), and deoxythymidine triphosphate (dTTP).

30. The method of claim 25 wherein said method is carried out at an excess concentration of said polynucleotide **primer** relative to the concentration of said polynucleotide.

31. The method of claim 25 wherein step (d) is repeated such that the number of molecules of said extended polynucleotide **primer** formed is increased by at least a factor of three.

32. The method of claim 25 wherein said polynucleotide is labeled with a reporter group.

33. The method of claim 32 wherein said reporter group is selected from the group consisting of, fluorescers, chemiluminescers, catalysts, co-enzymes, radioactive substances, amplifiable polynucleotide sequences, and small organic molecules.

34. The method of claim 25 wherein said polydeoxynucleotide **primer** is labeled with a ligand.

35. The method of claim 25 wherein said polynucleotide ligated to said polynucleotide analyte contains a sequence that when hybridized to its

36. The method of claim 35 wherein said receptor is selected from the group consisting of repressors, activators, and restriction enzymes.
37. The method of claim 25 wherein said polynucleotide ligated to said polynucleotide analyte contains a sequence at its end that when hybridized to its complementary sequence, can be bound specifically by a receptor, and said extended polydeoxynucleotide **primer** is detected by binding said receptor to said extended polynucleotide **primer**.
38. The method of claim 25 wherein the 5'-end of said polynucleotide analyte is separated from said segment by 10 nucleotides.
39. The method of claim 25 wherein the nucleotide at the 5'-end of said polynucleotide analyte is cytidine (C).
40. The method of claim 25 wherein each of said segments has a sequence of from 10 to 100 nucleotides hybridizable with the other.
41. The method of claim 25 wherein said polynucleotide is deoxyribonucleic acid (DNA).
42. The method of claim 1 wherein said T4 deoxyribonucleic acid ligase is present in a concentration of about 200 to 100 fold excess relative to the concentration of said first and second polynucleotide sequences.
43. The method of claim 14 wherein said T4 deoxyribonucleic acid ligase is present in a concentration of about 200 to 100 fold excess relative to the concentration of said polynucleotide and said target polynucleotide sequence.
44. The method of claim 24 wherein said T4 deoxyribonucleic acid ligase is present in a concentration of about 200 to 100 fold excess relative to the concentration of said polynucleotide and said polynucleotide analyte.
45. The method of claim 25 wherein said T4 deoxyribonucleic acid ligase is present in a concentration of about 200 to 100 fold excess relative to the concentration of said polynucleotide and said polynucleotide analyte.
46. The method of claim 25 wherein said first terminal nucleotide has a 5'-phosphate group and said second terminal nucleotide has a 3'-hydroxyl group.
47. The method of claim 15 wherein said first terminal nucleotide has a 3'-hydroxyl group and said second terminal nucleotide has a 5'-phosphate group.
48. The method of claim 15 wherein said polynucleotide **primer** is said polynucleotide.
49. The method of claim 25 wherein said polynucleotide **primer** is said polynucleotide.

L5 ANSWER 110 OF 112 USPATFULL on STN

95:22820 Amplification method for polynucleotide detection assays.

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US 5397698 19950314

APPLICATION: US 1993-146297 19931102 (8)

DOCUMENT TYPE: Utility; Granted.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method is disclosed for producing multiple copies of a primary polynucleotide sequence located at the 3' terminus of a polynucleotide. The method comprises (a) forming in the presence of nucleoside triphosphates and template-dependent polynucleotide polymerase an extension of a primary polynucleotide sequence hybridized with a template sequence of a single stranded pattern polynucleotide comprising two or more template sequences each containing one or more site specific cleavage sequences, (b) cleaving into fragments said extension at cleavable polynucleotide sequences in the presence of means for specifically cleaving said cleavable polynucleotide sequences when said extension is hybridized with said site specific cleavage sequences, (c) dissociating said fragments, (d) hybridizing said fragments with single stranded pattern polynucleotide, and repeating steps (a)-(d). Steps (a)-(d) may be conducted simultaneously or wholly or partially sequentially. The method may be applied in the detection of a

to facilitate such detection. Also disclosed are compositions for conducting the method of the invention.

What is claimed is:

1. A method of determining the presence of a polynucleotide analyte in a sample suspected of containing said analyte, which comprises: (a) forming in the presence of nucleoside triphosphates and template-dependent polynucleotide polymerase an extension of a polynucleotide analyte having a 3' hydroxy nucleotide, a part thereof including said 3' hydroxy nucleotide being hybridized with a binding polynucleotide sequence of a single stranded pattern polynucleotide comprising said binding polynucleotide sequence connected at its 5' end to two or more template sequences each containing one or more site specific cleavage sequences, (b) cleaving into fragments said extension at cleavable polynucleotide sequences in the presence of means for specifically cleaving said cleavable polynucleotide sequences when said extension is hybridized with said site specific cleavage sequences, (c) dissociating said fragments, (d) hybridizing said fragments with said single stranded pattern polynucleotide, (e) forming in the presence of said nucleoside triphosphates and said template dependent polynucleotide polymerase an extension of said fragments hybridized with said single stranded pattern polynucleotide, (f) repeating steps (b)-(e) above wherein steps (b)-(e) are conducted simultaneously or wholly or partially sequentially, and (g) detecting said fragments or fragments complementary thereto, the presence thereof indicating the presence of said polynucleotide analyte in said sample.

2. The method of claim 1 wherein said fragments are formed by a restriction enzyme and said single stranded pattern polynucleotide is DNA.

3. The method of claim 1 wherein said nucleotide triphosphates are selected from three members of the group consisting of dATP, dTTP, dGTP, and dCTP and derivatives thereof and said template sequences and the sequences connecting said template sequences consist of a continuous sequence of nucleotides selected from three members of the group consisting of A and dA, U and dT, C and dC, and G and dG and derivatives thereof that are complementary to said nucleoside triphosphates.

4. The method of claim 1 wherein prior to said forming said sample is incubated sequentially with (1) reagents for modifying the 3' end of any polynucleotide in said sample to prevent said 3' end from reacting with said template-dependent polynucleotide polymerase and (2) a restriction endonuclease capable of cleaving said polynucleotide analyte.

5. The method of claim 4 wherein said reagents comprise an enzyme capable of catalyzing a reaction of a polynucleotide 3'-hydroxyl group.

6. The method of claim 4 wherein said reagents are a ligase and an **oligonucleotide** terminated at the 3' end by a group that does not react to provide chain extension by said template-dependent polynucleotide polymerase.

7. The method of claim 4 wherein said reagents are a terminal transferase and a dideoxynucleoside triphosphate.

8. The method of claim 1 for determining the presence of a RNA analyte in a sample suspected of containing said RNA analyte, wherein said polynucleotide analyte terminating in a 3'-hydroxy nucleotide is provided by: combining in an aqueous medium said sample, a single stranded DNA **primer** comprising a deoxynucleic acid sequence containing a restriction site and capable of hybridizing with said RNA sequence, and a restriction enzyme capable of cleaving said **primer** at said restriction site when said **primer** is hybridized with said RNA sequence, and incubating said medium for a time sufficient to permit cleaving to occur.

9. The method of claim 8 wherein said **primer** is at least a portion of a cyclic polynucleotide or is terminated at its 3' end by a group incapable of reacting in a reaction catalyzed by said template-dependent polynucleotide polymerase.

10. A method for detecting the presence of a polynucleotide analyte in a sample suspected of containing said polynucleotide analyte which comprises: combining either simultaneously or wholly or partially sequentially (1) said sample, (2) means for causing said polynucleotide analyte to be terminated in a 3'--OH group when said analyte is not terminated in a 3'--OH group, (3) a single stranded pattern polynucleotide comprising a binding polynucleotide sequence hybridizable with said polynucleotide analyte and connected to the 3' end of a strand of two or more template sequences connected in tandem and containing site specific cleavage sequences, (4) nucleoside triphosphates, (5) template-dependent polynucleotide polymerase, and (6) means for

to said site specific cleavage sequences when said cleavable polynucleotide sequences are hybridized with said site specific cleavage sequences and incubating said combination under conditions for either simultaneously or wholly or partially sequentially (a) causing said polynucleotide analyte to be terminated in a 3'--OH group when said analyte is not terminated in a 3'--OH group, (b) hybridizing said polynucleotide analyte with said single stranded pattern polynucleotide, (c) forming an extension of said polynucleotide analyte comprising a sequence complementary to said template sequence and to said site specific cleavage sequences, (d) cleaving said extension into fragments at said cleavable polynucleotide sequences, (e) dissociating said fragments, (f) hybridizing said dissociated fragments with single stranded pattern polynucleotide, and (g) forming an extension of said hybridized fragments along said pattern polynucleotide, and repeating steps (d)-(g), and determining the presence of said fragments, or fragments complementary thereto, the presence thereof indicating the presence of said polynucleotide analyte in said sample.

11. The method of claim 10 wherein said site specific cleavage sequences are cleaved.

12. The method of claim 11 wherein said fragments include those of the single stranded pattern polynucleotide.

13. The method of claim 10 wherein said polynucleotide analyte is DNA.

14. The method of claim 10 wherein said single stranded pattern polynucleotide is DNA.

15. The method of claim 14 wherein said single stranded pattern polynucleotide comprises at least three of said template sequences.

16. The method of claim 15 wherein said site specific cleavage sequences contained in said template sequences are all identical.

17. The method of claim 14 wherein said single stranded pattern polynucleotide is terminated at the 3' end by a group that is incapable of reacting in a reaction catalyzed by said template dependent polynucleotide polymerase.

18. The method of claim 10 wherein said template sequence is 8 to 100 nucleotides in length.

19. The method of claim 10 wherein said site specific cleavage sequence is a restriction endonuclease site and said means for specifically cleaving said cleavable polynucleotide sequences comprises a restriction endonuclease.

20. The method of claim 10 wherein said template-dependent polynucleotide polymerase is a DNA polymerase and said nucleoside triphosphates are ATP, GTP, CTP, and TTP.

21. The method of claim 10 wherein said incubation is carried out at a substantially constant temperature.

22. The method of claim 10 wherein the number of said fragments formed for each of said extensions is increased at least by a factor of three.

23. The method of claim 10 wherein said polynucleotide analyte is RNA.

24. The method of claim 10 wherein said single stranded pattern polynucleotide is at least part of a cyclic polynucleotide.

25. A method for determining the presence of a polynucleotide analyte in a sample suspected of containing said polynucleotide analyte, which method comprises: combining in an aqueous medium either simultaneously or wholly or partially sequentially (1) said sample (2) means for obtaining from said polynucleotide analyte a target sequence and for terminating said sequence in a 3'--OH group when said sequence is not terminated in a 3'--OH group, (3) single stranded pattern polydeoxynucleotide comprised of a binding polynucleotide sequence complementary to said target sequence joined at its 5' end to a multiply repeated sequence of at least 10 nucleotides, said repeated sequence, when hybridized to its complementary sequence and incubated with a restriction endonuclease, promoting cleavage of said complementary sequence to form restriction fragments, (4) deoxynucleoside triphosphates, (5) a DNA dependent DNA polymerase for extending said target sequence to provide said complementary sequence, and (6) a restriction endonuclease for forming said restriction fragments, said combining being carried out under conditions for either simultaneously or wholly or partially sequentially (a) obtaining said target sequence from said polynucleotide analyte, (b) hybridizing said target sequence

target sequence to form said complementary sequence, (d) cleaving said complementary sequence, (e) denaturing the duplex of said complementary sequence and said repeated sequence, (f) hybridizing said complementary sequence with single stranded pattern polydeoxynucleotide, (g) extending said complementary sequence, and repeating steps (d)-(g) above; and detecting said cleaved complementary sequence, the presence thereof indicating the presence of said polynucleotide analyte in said sample.

26. The method of claim 25 wherein said repeated sequence is cleaved by said restriction endonuclease.

27. The method of claim 26 wherein detecting said cleaved complementary sequence includes detecting said cleaved repeated sequence.

28. The method of claim 25 wherein said analyte is DNA and said means for obtaining a target sequence includes a restriction enzyme.

29. The method of claim 25 wherein said analyte is RNA and said means for obtaining a target sequence includes (1) a single stranded DNA **primer** comprising a deoxynucleic acid sequence capable of hybridizing with said target RNA sequence and containing a restriction site, said **primer** being terminated at its 3' end by a group that prevents chain extension by DNA-dependent DNA polymerase and (2) a restriction endonuclease capable of cleaving said **primer** at said restriction site when said **primer** is hybridized with said RNA sequence and said aqueous medium is incubated for a time sufficient to permit said RNA analyte to hybridize with said DNA **primer** and to permit said restriction endonuclease to cleave the hybridized RNA analyte-DNA **primer**.

30. The method of claim 25 wherein said repeated sequence contains 12 to 100 nucleotides.

31. The method of claim 25 wherein said deoxynucleoside triphosphates are dATP, dGTP, dCTP, and dTTP.

32. The method of claim 25 wherein said conditions include substantially constant temperature.

33. The method of claim 25 wherein said deoxynucleoside triphosphates are selected from three members of the group consisting of dATP, dTTP, dGTP, and dCTP and derivatives thereof.

34. The method of claim 33 wherein said template sequences and polydeoxynucleotide sequences joining said templates sequences consist of a continuous sequence of nucleotides selected from three members of the group consisting of dA, dT, dC, dG and derivatives thereof.

35. The method of claim 25 wherein said cleaved complementary sequence is determined by a method selected from the group consisting of nucleic acid probe hybridization, spectroscopic detection and chromatographic detection.

36. A method for determining the presence of a target sequence of nucleotides in a polynucleotide analyte in a sample suspected of containing said polynucleotide analyte, which method comprises: combining either simultaneously or wholly or partially sequentially said sample and (1) means capable of causing said target sequence to be terminated in a 3'--OH group when said target sequence is not already terminated in a 3'--OH group, (2) single stranded pattern polydeoxynucleotide comprised of a sequence complementary to said target sequence, said sequence bonded at its 5' end to a multiply repeating sequence of at least 10 nucleotides which, when hybridized to a complementary sequence, promotes cleavage of said complementary sequence to form fragments (3) deoxynucleoside triphosphates, (4) DNA dependent DNA polymerase and (5) a restriction endonuclease for cleaving said complementary sequence when hybridized to said multiply repeating sequence; incubating the individual components and mixtures formed therefrom under conditions which promote either wholly or partially sequential or simultaneous (a) denaturation of said target sequence when said target sequence is double stranded, (b) hybridization of said target sequence with said single stranded pattern polydeoxynucleotide, (c) extension of said target sequence by DNA dependent DNA polymerase to produce a duplex containing said complementary sequence, (d) cleavage of said complementary sequence in said duplex, (e) melting of said duplex, (f) hybridization of said cleaved complementary sequence, (g) extension of said complementary sequence by DNA dependent DNA polymerase to produce a duplex containing said complementary sequence, and repeating steps (d)-(g) above; and detecting said complementary sequence or fragments of said pattern polydeoxynucleotide produced during cleavage of said complementary sequence, the presence thereof indicating the presence of said analyte in said sample.

means capable of causing said target sequence to terminate in a 3'--OH group includes a restriction enzyme.

38. The method of claim 36 wherein said complementary sequence contains 10 to 100 nucleotides.

39. The method of claim 36 wherein said deoxynucleoside triphosphates are dATP, dGTP, dCTP and dTTP.

40. The method of claim 36 wherein said conditions include substantially constant temperature.

41. The method of claim 36 wherein said deoxynucleoside triphosphates are selected from three members of the group consisting of dATP, dTTP, dGTP, and dCTP and derivatives thereof.

42. The method of claim 41 wherein said template sequences and polydeoxynucleotide sequences joining said template sequences consist of a continuous sequence of nucleotides selected from three members of the group consisting of dA, dT, dC, and dG, and derivatives thereof.

L5 ANSWER 111 OF 112 USPATFULL on STN

93:108981 Amplification method for polynucleotide assays.

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US 5273879 19931228

APPLICATION: US 1990-614180 19901113 (7)

DOCUMENT TYPE: Utility; Granted.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A Kit is disclosed for a method for producing multiple copies of a primary polynucleotide sequence located at the 3' terminus of a polynucleotide. The method comprises (a) forming in the presence of nucleoside triphosphates and template-dependent polynucleotide polymerase an extension of a primary polynucleotide sequence hybridized with a template sequence of a single stranded pattern polynucleotide comprising two or more template sequences each containing one or more site specific cleavage sequences, (b) cleaving into fragments said extension at cleavable polynucleotide sequences in the presence of means for specifically cleaving said cleavable polynucleotide sequences when said extension is hybridized with said site specific cleavage sequences, (c) dissociating said fragments, (d) hybridizing said fragments with single stranded pattern polynucleotide, and repeating steps (a)-(d). Steps (a)-(d) may be conducted simultaneously or wholly or partially sequentially. The method may be applied in the detection of a polynucleotide analyte in a sample suspected of containing such analyte to facilitate such detection. Also disclosed are compositions for conducting the method of the invention.

CLM What is claimed is:

1. A kit comprising (a) a single stranded DNA oligomer bonded at its 3' end to a single stranded polynucleotide binding sequence wherein said binding sequence is complementary to a polynucleotide target sequence comprising 12 to 1000 nucleotides, wherein said oligomer consists of about 3 to 100 **oligonucleotide** units each consisting of an identical **oligonucleotide** template sequence having about 8 to 100 nucleotides and at least one restriction site when said template sequence is hybridized to a complementary sequence, wherein said oligomer is consists of only three different nucleotides, said nucleotides being selected from the group consisting of dATP, dTTP, dGTP and dCTP and derivatives thereof, (b) deoxynucleoside triphosphates, (c) DNA-dependent DNA polymerase, (d) restriction endonuclease capable of cleaving said restriction site.

2. The kit of claim 1 which further comprises in combination a single stranded DNA **primer** comprising a nucleic acid sequence capable of hybridizing with RNA and containing a restriction site, said **primer** being terminated at its 3' end by a group incapable of reacting in a chain extension by said DNA-dependent DNA polymerase.

3. The kit of claim 2 which further comprises a restriction enzyme capable of cleaving said **primer** at said restriction site when said **primer** is hybridized with said RNA sequence.

L5 ANSWER 112 OF 112 USPATFULL on STN

91:15075 Amplification method for polynucleotide assays.

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APPLICATION: US 1987-76807 19870723 (7)

DOCUMENT TYPE: Utility; Granted.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method is disclosed for producing multiple copies of a primary polynucleotide sequence located at the 3' terminus of a polynucleotide. The method comprises (a) forming in the presence of nucleoside triphosphates and template-dependent polynucleotide polymerase an extension of a primary polynucleotide sequence hybridized with a template sequence of a single stranded pattern polynucleotide comprising two or more template sequences each containing one or more site specific cleavage sequences, (b) cleaving into fragments said extension at cleavable polynucleotide sequences in the presence of means for specifically cleaving said cleavable polynucleotide sequences when said extension is hybridized with said site specific cleavage sequences, (c) dissociating said fragments, (d) hybridizing said fragments with single stranded pattern polynucleotide, and repeating steps (a)-(d). Steps (a)-(d) may be conducted simultaneously or wholly or partially sequentially. The method may be applied in the detection of a polynucleotide analyte in a sample suspected of containing such analyte to facilitate such detection. Also disclosed are compositions for conducting the method of the invention.

CLM What is claimed is:

1. A method of producing multiple copies of a primary polynucleotide sequence located at the 3' terminus of a polynucleotide, which comprises: (a) forming in the presence of nucleoside triphosphates and template-dependent polynucleotide polymerase an extension of a primary polynucleotide sequence hybridized with a template sequence of a single stranded pattern polynucleotide comprising two or more identical template sequences each containing one or more site specific cleavage sequences, (b) cleaving into fragments said extension at cleavable polynucleotide sequences in the presence of means for specifically cleaving said cleavable polynucleotide sequences when said extension is hybridized with said site specific cleavage sequences, (c) dissociating said fragments, said fragments comprising a primary polynucleotide sequence, (d) hybridizing said fragments with said single stranded pattern polynucleotide, and repeating steps (a)-(d) above wherein steps (a)-(d) are conducted simultaneously or wholly or partially sequentially.

2. The method of claim 1 wherein said primary polynucleotide sequence is DNA.

3. The method of claim 1 wherein said single stranded pattern polynucleotide comprises an oligomer of at least three of an identical template sequence.

4. The method of claim 3 wherein said single stranded pattern polynucleotide is cyclic.

5. The method of claim 3 wherein said site specific cleavage sequences contained in said template sequences are all identical.

6. The method of claim 3 wherein said oligomer is terminated at the 3' end by a chemical group or solid support incapable of reacting in a chain extension reaction of said oligomer catalyzed by said template dependent polynucleotide polymerase.

7. The method of claim 1 wherein said template sequence is 8 to 100 nucleotides in length.

8. The method of claim 1 wherein said site specific cleavage sequence is a restriction endonuclease site and said means for specifically cleaving said cleavable polynucleotide sequences comprises a restriction endonuclease.

9. The method of claim 1 wherein said template-dependent polynucleotide polymerase is a DNA polymerase and said deoxynucleoside triphosphates are dATP, dGTP, dCTP, and dTTP.

10. The method of claim 1 wherein said method is carried out at a substantially constant temperature.

11. A method of producing multiple copies of a primary polynucleotide sequence located at the 3' terminus of a polynucleotide, which comprises: (a) forming in the presence of nucleoside triphosphates and template-dependent polynucleotide polymerase an extension of a primary polynucleotide sequence hybridized with a template sequence of a single stranded pattern polynucleotide comprising two or more template sequences each containing one or more site specific cleavage sequences. (b) cleaving into fragments said extension at cleavable polynucleotide

cleavable polynucleotide sequences when said extension is hybridized with said site specific cleavage sequences. (c) dissociating said fragments, said fragments comprising a primary polynucleotide sequence. (d) hybridizing said fragments with said single stranded pattern polynucleotide, and repeating steps (a)-(d) above wherein steps (a)-(d) are conducted simultaneously or wholly or partially sequentially and wherein the number of said copies of said primary polynucleotide sequence formed for each of said extension is increased by at least a factor of three.

12. The method of claim 1 wherein the 3' end of said template sequences in said single stranded pattern polynucleotide is connected to a polynucleotide sequence complementary to a polynucleotide analyte in a sample suspected of containing said polynucleotide analyte.

13. The method of claim 1 wherein said single stranded pattern polynucleotide is at least part of a cyclic polynucleotide.

14. The method of claim 1 wherein said nucleoside triphosphates are selected from three members of the group consisting of dATP, dTTP, dGTP, and dCTP and derivatives thereof.

15. The method of claim 1 wherein said template sequences and the sequences connecting said template sequences consist of a continuous sequence of nucleotides selected from three members of the group consisting of A and dA, U and dT, C and dC, and G and dG and derivatives thereof.

16. The method of claim 15 wherein said derivatives are selected from the group consisting of methylated nucleotides and nucleotides labeled with a reporter group.

17. The method of claim 16 wherein said reporter group is selected from the group consisting of biotin, fluorescers, chemilumescers, and small organic molecules.

18. A method of producing multiple copies of a primary polynucleotide sequence as the result of the presence of a target polynucleotide sequence located at the 3' terminus of a polynucleotide, which comprises: (a) forming in the presence of nucleoside triphosphates and template-dependent polynucleotide polymerase an extension of a target polynucleotide sequence hybridized with a binding polynucleotide sequence of a single stranded pattern polynucleotide comprising said binding polynucleotide sequence and two or more copies of a template sequence each containing one or more site specific cleavage sequences, (b) cleaving into fragments said extension at cleavable polynucleotide sequences in the presence of means for specifically cleaving said cleavable polynucleotide sequences when said extension is hybridized with said site specific cleavage sequences, (c) dissociating said fragments, wherein said fragments comprise said primary polynucleotide sequence (d) hybridizing said fragments with said single stranded pattern polynucleotide, (e) forming in the presence of said nucleoside triphosphates and said template dependent polynucleotide polymerase an extension of said fragments hybridized with said single stranded pattern polynucleotide, and (f) repeating steps (b)-(e) above wherein steps (b)-(e) are conducted simultaneously or wholly or partially sequentially.

19. The method of claim 18 wherein said target polynucleotide sequence is DNA.

20. The method of claim 18 wherein said single stranded pattern polynucleotide comprises an oligomer of at least three of said template sequences.

21. The method of claim 18 wherein said single stranded pattern polynucleotide is cyclic.

22. The method of claim 18 wherein said site specific cleavage sequences contained in said template sequences are all identical.

23. The method of claim 18 wherein said binding polynucleotide sequence is connected to said template sequence by a site specific cleavage sequence.

24. The method of claim 20 wherein said oligomer is terminated at the 3' end by a group incapable of reacting in a chain extension reaction of said oligomer catalyzed by said template dependent polynucleotide polymerase.

25. The method of claim 18 wherein said template sequence is 8 to 100 nucleotides in length.

26. The method of claim 18 wherein said site specific cleavage sequence is a restriction endonuclease site and said means for specifically cleaving said cleavable polynucleotide sequences comprises a restriction endonuclease.

27. The method of claim 18 wherein said template-dependent polynucleotide polymerase is a DNA polymerase and said nucleoside triphosphates are dATP, dGTP, dCTP, and dTTP.

28. The method of claim 18 wherein said method is carried out at a substantially constant temperature.

29. The method of claim 18 wherein said single stranded pattern polynucleotide comprises 5 to 50 template sequences connected by one or more site specific cleavage sequences.

30. A method of producing multiple polynucleotide molecules as a function of the presence of a target sequence of nucleotides present in a polynucleotide sample, which method comprises: combining either wholly or partially sequentially or simultaneously (1) said polynucleotide sample containing said target sequence, (2) means to cause said target sequence to terminate in a free 3'--OH group when said target sequence does not already terminate in a free 3'-OH group, (3) single stranded pattern polynucleotide comprising a binding polynucleotide sequence complementary to substantially all of said target sequence, joined at its 5' end to two or more identical template sequences each containing site specific cleavage sequence, (4) nucleoside triphosphates, (5) template-dependent polynucleotide polymerase, and (6) means for cleaving a sequence complementary to said site specific cleavage sequences when hybridized with said site specific cleavage sequence, said combining being carried out under conditions which promote either wholly or partially sequentially or simultaneously (a) denaturation of said target sequence when said target sequence is double stranded, (b) hybridization of said target sequence with said binding polynucleotide sequence, (c) extension of said target sequence by template dependent polymerization to produce a duplex, (d) cleavage of said extension in said duplex into fragments, (e) reversible denaturation of said fragments, (f) hybridization of said fragments with single stranded pattern polynucleotide, and (g) extension of said hybridized fragments by template dependent polymerization to produce a duplex, and repeating steps (d)-(g).

31. The method of claim 30 wherein said single stranded pattern polynucleotide is present in excess amount over the amount of said target sequence and said conditions include an essentially constant temperature.

32. The method of claim 30 wherein said binding sequence and said template sequence are the same.

33. The method of claim 30 wherein said polynucleotide sample is DNA and said means to cause said target sequence to terminate in a 3'--OH group is a restriction enzyme.

34. The method of claim 30 wherein said site specific cleavage sequence in said single stranded pattern polynucleotide is a sequence capable of cleavage by a restriction endonuclease.

35. The method of claim 34 wherein said restriction endonuclease is the same as the means to cause said target sequence to terminate in a 3'--OH group.

36. The method of claim 30 wherein said site specific cleavage sequence includes a sequence complementary to the target sequence.

37. The method of claim 30 wherein the 5' end of said template sequence is bonded with or without an intervening polynucleotide sequence to the 3' end of said binding sequence to form a ring.

38. The method of claim 30 wherein said template sequence is about 8-100 nucleotides long and said single stranded pattern polynucleotide comprises an oligomer containing 2-100 of said template sequences.

39. The method of claim 30 wherein said single stranded pattern polynucleotide is terminated at the 3' end by a group that is incapable of reacting in a reaction catalyzed by said template dependent polynucleotide polymerase.

40. The method of claim 30 wherein said polynucleotide sample is RNA and said means to cause said target sequence to terminate in a 3'--OH group includes (1) a single stranded DNA **primer** comprising a deoxynucleic acid sequence containing a restriction site and capable of hybridizing

cleaving said **primer** at said restriction site when said **primer** is hybridized with said RNA sequence.

41. The method of claim 40 wherein said **primer** is cyclic or is terminated at its 3' end by a group incapable of reacting in a reaction catalyzed by said template-dependent polynucleotide polymerase.

42. The method of claim 30 wherein said polynucleotide sample is selected from the group consisting of the genomes of viruses, bacteria, molds, and fungi, and fragments thereof.

43. The method of claim 30 wherein said template dependent polynucleotide polymerase is DNA polymerase.

44. The method of claim 30 wherein said single stranded pattern polynucleotide is a linear **oligonucleotide**.

45. The method of claim 30 wherein said single stranded pattern polynucleotide is at least part of a cyclic polynucleotide.

46. The method of claim 30 wherein said polynucleotide sample is double stranded DNA and said target sequence is caused to terminate in a free 3'--OH group by cleaving said DNA with a restriction endonuclease.

47. The method of claim 30 wherein said polynucleotide sample is a DNA-RNA heteroduplex formed by hybridizing RNA with a single stranded oligodeoxynucleotide complementary to said binding polynucleotide sequence and terminated at the 3' end by a group that is incapable of reacting in a reaction catalyzed by said template-dependent polynucleotide polymerase, said heteroduplex providing for cleavage by a restriction endonuclease of at least said oligodeoxynucleotide with formation of said target sequence terminating in a 3'-hydroxyl.

48. The method of claim 30 wherein terminal 3'--OH groups of said polynucleotide sample are rendered unreactive with said template dependent polynucleotide polymerase prior to combining said polynucleotide sample with said means to cause said target sequence to terminate in a 3'--OH group.

49. The method of claim 30 wherein said nucleoside triphosphates are selected from three members of the group consisting of dATP, dTTP, dGTP, and dCPT and derivatives thereof.

50. The method of claim 49 wherein said template sequence and polynucleotide sequences joining said template sequences consists of a continuous sequence of nucleotides selected from three members of the group consisting of A and dA, U and dT, C and dC, G and dG and derivatives thereof.

51. A method of producing multiple copies of a primary polynucleotide sequence located at the 3' terminus of a polynucleotide which comprises: providing in combination either wholly or partially sequentially or concomitantly (1) said primary polynucleotide sequence hybridized with single stranded pattern polynucleotide, (2) single stranded pattern polynucleotide comprising two or more identical template sequences each containing site specific cleavage sequences, (3) nucleoside triphosphates, (4) template-dependent polynucleotide polymerase, and (5) means for specifically cleaving cleavable polynucleotide sequences complementary to site specific cleavage sequences when said cleavable polynucleotide sequences are hybridized with said site specific cleavage sequences and incubating said combination under conditions for either wholly or partially sequentially or concomitantly (a) forming an extension of said primary polynucleotide sequence comprising one or more copies thereof, (b) cleaving said extension at said cleavable polynucleotide sequences into primary polynucleotide sequences, (c) dissociating said hybridized extension, (d) hybridizing said primary polynucleotide sequences with said single stranded pattern polynucleotide, and repeating at least steps (a) and (b) above.

52. A method of producing multiple copies of a primary polynucleotide sequence as the result of the presence of a target polynucleotide sequence located at the 3' terminus of a polynucleotide which comprises: providing in combination either wholly or partially sequentially or concomitantly (1) said target polynucleotide sequence hybridized to a single stranded pattern polynucleotide comprising a binding sequence complementary to said target polynucleotide sequence and one or more copies of a template sequence complementary to said primary polynucleotide sequence each containing two or more site specific cleavage sequences, (2) single stranded pattern polynucleotide, (3) nucleoside triphosphates, (4) template-dependent polynucleotide polymerase, and (5) means for specifically cleaving cleavable polynucleotide sequences complementary to said site specific cleavage

with said site specific cleavage sequences and incubating said combination under conditions for either wholly or partially sequentially or concomitantly (a) forming an extension of said target sequence comprising one or more of said copies of said primary polynucleotide sequence connected through said cleavable polynucleotide sequences, (b) cleaving said extension at said cleavable polynucleotide sequences into said target sequence and primary polynucleotide sequences, (c) dissociating said hybridized extension, (d) hybridizing said primary polynucleotide sequence with said single stranded pattern polynucleotide, and (e) forming an extension of said primary polynucleotide sequence comprising one or more copies of said primary polynucleotide sequence, and repeating steps (b)-(e).

53. A method for producing multiple copies of a primary polydeoxynucleotide sequence complementary to a template sequence, which comprises: providing in an aqueous medium either simultaneously or wholly or partially sequentially a mixture comprising (1) said primary polydeoxynucleotide sequence; (2) a cyclic single stranded pattern polydeoxynucleotide comprising an oligomer of at least three identical template sequences each containing one or more restriction endonuclease sites, said oligomer consisting of only three independent nucleotides selected from the group consisting of deoxynucleotides or corresponding derivatives thereof; (3) the three deoxynucleotide triphosphates complementary to said deoxynucleotides corresponding to the bases in said primary polydeoxynucleotide sequence; (4) DNA dependent DNA polymerase; and (5) one or more restriction endonucleases capable of cleaving a duplex consisting of said single stranded pattern polydeoxynucleotide and single stranded DNA complementary to said pattern polydeoxynucleotide, said cleaving occurring at said restriction sites and only when said single stranded pattern polydeoxynucleotide is bound to said complementary single stranded DNA; and incubating said mixture under conditions for either simultaneously or wholly or partially sequentially hybridizing said primary polydeoxynucleotide with said single stranded pattern polydeoxynucleotide, extending said primary polydeoxynucleotide along said single stranded pattern polynucleotide to form said duplex, cleaving said duplex at said restriction sites, dissociating said primary polydeoxynucleotide from said template sequence, and rehybridizing said primary polydeoxynucleotide with said single stranded pattern polydeoxynucleotide.

54. The method of claim 53 wherein said primary polydeoxynucleotide sequence is formed in response to the presence of a polynucleotide analyte in a sample suspected of containing said polynucleotide analyte which further comprises the steps of: prior to said providing (1) treating said polynucleotide analyte to provide a single stranded target sequence terminating in a 3'-hydroxy group, (2) incubating said target sequence with reagents required to provide said primary polydeoxynucleotide sequence, and subsequent to said incubating (3) detecting said multiple copies of said primary polydeoxynucleotide sequence.

55. The method of claim 54 wherein said polynucleotide analyte is treated in step (1) with a restriction endonuclease to provide said 3'-hydroxy group.

56. The method of claim 55 wherein said polynucleotide analyte is first denatured and then incubated with a single stranded binding polydeoxynucleotide sequence containing a restriction site recognized by said endonuclease.

57. The method of claim 56 wherein said single stranded binding polydeoxynucleotide sequence is a part of said single stranded pattern polydeoxynucleotide.

58. The method of claim 54 wherein said single stranded pattern polydeoxynucleotide further comprises a binding polydeoxynucleotide sequence complementary to said target sequence and connected at its 5' end to the 3' end of said oligomer only by combinations of said three deoxynucleotides.

59. The method of claim 54 wherein said reagents for incubating said target sequence to provide said primary polydeoxynucleotide sequence include (1) a binding polynucleotide sequence complementary to said target sequence connected at its 5' end to the 3' end of said template sequence either directly or by a combination of said three nucleotides, and (2) a restriction endonuclease capable of cleaving at said cleavage site.

60. A method of producing multiple copies of a primary polydeoxynucleotide sequence complementary to a template sequence which comprises: (a) forming in the presence of three members selected from the group consisting of deoxynucleoside triphosphates or corresponding

primary polydeoxynucleotide sequence hybridized with a template sequence of a cyclic single stranded pattern polydeoxynucleotide comprising an oligomer having at least three identical template sequences each containing one or more restriction endonuclease sites and a sequence of nucleotides complementary to said three members and derivatives thereof, (b) cleaving into fragments said extension at said restriction endonuclease sites in the presence of one or more restriction endonucleases capable of cleaving at said restriction endonuclease sites only when said extension is hybridized with said single stranded pattern polynucleotide, (c) dissociating said fragments, (d) hybridizing said fragments with said single stranded pattern polydeoxynucleotide, and repeating steps (a)-(d) above wherein steps (a)-(d) are conducted simultaneously or wholly or partially sequentially.

61. The method of claim 60 wherein said primary polydeoxynucleotide sequence is formed in response to the presence of a polynucleotide analyte in a sample suspected of containing said polynucleotide analyte wherein prior or simultaneously with said forming (1) said polynucleotide analyte is treated to provide a single stranded target sequence terminating in a 3'-hydroxy group and (2) said target sequence is incubated with reagents required to provide said primary polydeoxynucleotide sequence, and (3) wherein subsequent to said repeating said multiple copies of said primary polydeoxynucleotide sequence are detected.

62. The method of claim 61 wherein said polynucleotide analyte is treated in step (1) with a restriction endonuclease to provide said 3'-hydroxy group.

63. The method of claim 62 wherein said polynucleotide analyte is first denatured and then incubated with a single stranded binding polydeoxynucleotide sequence containing a restriction site recognized by said endonuclease.

64. The method of claim 63 wherein said single stranded binding polydeoxynucleotide sequence is a part of said single stranded pattern polydeoxynucleotide.

65. The method of claim 61 wherein said single stranded pattern polydeoxynucleotide further comprises a binding polydeoxynucleotide sequence complementary to said target sequence and connected at its 5' end to the 3' end of said oligomer only by combinations of said three deoxynucleotides.

66. The method of claim 61 wherein said reagents for incubating said target sequence to provide said primary polydeoxynucleotide sequence include (1) a binding polynucleotide sequence complementary to said target sequence connected at its 5' end to the 3' end of said template sequence either directly or by a combination of said nucleotides complementary to said three members, and (2) a restriction endonuclease capable of cleaving at said cleavage site.

67. A method for producing multiple copies of a primary polynucleotide sequence as the result of the presence of a target polynucleotide sequence, which method comprises: (a) combining (1) a single stranded pattern polynucleotide comprising a binding sequence substantially complementary to said target polynucleotide sequence and two or more identical template sequences each containing one or more restriction sites with (2) said target polynucleotide sequence, (b) incubating said combination at a temperature that causes said target polynucleotide sequence to anneal selectively with said binding sequence, (c) adding to said combination template-dependent polynucleotide polymerase, a restriction endonuclease for at least one of said restriction sites in said template sequence and, if not already present, nucleoside triphosphates, (d) incubating said combination at a temperature sufficient to achieve chain extension of said target polynucleotide sequence and cleavage of said single stranded polynucleotide into fragments, (e) denaturing said fragments, said denatured fragments including said primary polynucleotide sequence, (f) incubating said combination at a temperature sufficient to anneal said primary polynucleotide sequence to said single stranded pattern polynucleotide, and (g) repeating steps (c)-(f) above.

68. A method for producing multiple copies of a sequence of nucleotides, which comprises hybridizing a **primer** polynucleotide sequence to a complementary polynucleotide sequence, said complementary polynucleotide sequence being part of a polynucleotide sequence having multiply repeating units complementary to said sequence of nucleotides to be copied, said multiply repeating units containing only two or three of the nucleotides A and dA or a derivative thereof, C and dC or a derivative thereof, U and dT or a derivative thereof, and G and dG or a derivative thereof, combining the hybridized material with only those

and with a polynucleotide polymerase, and incubating said combination under conditions for forming an extension of said **primer** polynucleotide sequence, wherein the above steps are carried out simultaneously or wholly or partially sequentially.

=> file wpids

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FULL ESTIMATED COST	382.01	382.22

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http://www.stn-international.de/stndatabases/details/ipc_reform.html and
<http://scientific.thomson.com/media/scpdf/ipcrdwpf.pdf> <<<

=> e wang wei kung/in

E1	6	WANG W Y/IN
E2	1	WANG WB/IN
E3	0 -->	WANG WEI KUNG/IN
E4	4672	WANG X/IN
E5	2	WANG X A/IN
E6	10	WANG X B/IN
E7	22	WANG X C/IN
E8	1	WANG X C H/IN
E9	1	WANG X C S/IN
E10	1	WANG X CH/IN
E11	6	WANG X D/IN
E12	1	WANG X E/IN

=> e wang w k/in

E1	11	WANG W J/IN
E2	1	WANG W J R/IN
E3	11 -->	WANG W K/IN
E4	8	WANG W L/IN
E5	1	WANG W L T/IN
E6	9	WANG W M/IN
E7	28	WANG W N/IN
E8	7	WANG W P/IN
E9	2	WANG W Q/IN
E10	3	WANG W S/IN
E11	15	WANG W T/IN
E12	1	WANG W T A/IN

=> s e3

L6 11 "WANG W K"/IN

=> d l6,bib,ab,1-11

L6 ANSWER 1 OF 11 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
Full Text
AN 2003-598314 [56] WPIDS
DNC C2003-162382

comprises reacting a disulfide-containing linker with an antibody to form modified antibody, removing unreacted linker, conjugating in solvent and purifying.

DC B04 D16

IN MAZZOLA, G L; **WANG, W K**; ZAPATA, G A; MAZZOLA, G J

PA (SMIK) SMITHKLINE BEECHAM CORP; (MAZZ-I) MAZZOLA G J; (WANG-I) WANG W K; (ZAPA-I) ZAPATA G A

CYC 101

PI WO 2003057163 A2 20030717 (200356)* EN 9

RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IT KE LS
LU MC MW MZ NL OA PT SD SE SI SK SL SZ TR TZ UG ZM ZW

W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK
DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR
KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT
RO RU SD SE SG SK SL TJ TM TN TR TT TZ UA UG US UZ VN YU ZA ZM ZW

AU 2003201824 A1 20030724 (200421)

EP 1467758 A2 20041020 (200469) EN

R: AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LI LT LU LV
MC MK NL PT RO SE SI SK TR

US 2005031627 A1 20050210 (200512)

JP 2005532258 W 20051027 (200571) 14

ADT WO 2003057163 A2 WO 2003-US205 20030102; AU 2003201824 A1 AU 2003-201824 20030102; EP 1467758 A2 EP 2003-700684 20030102, WO 2003-US205 20030102; US 2005031627 A1 WO 2003-US205 20030102, US 2004-500533 20040630; JP 2005532258 W JP 2003-557522 20030102, WO 2003-US205 20030102

FDT AU 2003201824 A1 Based on WO 2003057163; EP 1467758 A2 Based on WO 2003057163; JP 2005532258 W Based on WO 2003057163

PRAI US 2002-345305P 20020103

AB WO2003057163 A UPAB: 20030903

NOVELTY - Conjugating (M1) a maytansinoid (I) to an antibody (Ab) comprising:

(a) reacting a disulfide-containing linker with the Ab at pH 5-8 to form a modified Ab (II);

(b) removing unreacted linker from (II) by tangential flow filtration;

(c) conjugating (II) with (I) at pH 6-6.5 in a solvent comprising dimethylacetamide or acetonitrile; and

(d) purifying the (II)-maytansinoid conjugate by ion exchange chromatography.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for an antibody-mayansinoid conjugate prepared using M1.

ACTIVITY - Cytostatic. No biological data given.

MECHANISM OF ACTION - None given.

USE - M1 is used for conjugation of maytansinoid to an antibody (claimed) useful as anti-mitotic drugs and as immuno-conjugates for targeting tumor cells.

ADVANTAGE - The process is scalable, economical with improvements in efficiency, yield and productivity resulting in lower drug product manufacturing costs.

Dwg.0/1

L6 ANSWER 2 OF 11 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN

Full Text

AN 2003-503245 [47] WPIDS

DNN N2003-399483

TI Optical communication network includes multiple transmitters which emit optical signals at different output parameter values selected in accordance with gain profile of amplifiers.

DC V07 W02

IN KOSTE, G P; LIN, P J; **WANG, W K**

PA (KOST-I) KOSTE G P; (LINP-I) LIN P J; (WANG-I) WANG W K

CYC 1

PI US 2003011853 A1 20030116 (200347)* 11

ADT US 2003011853 A1 Provisional US 2001-268441P 20010213, US 2002-75067 20020212

PRAI US 2001-268441P 20010213; US 2002-75067 20020212

AB US2003011853 A UPAB: 20030723

NOVELTY - Multiple amplifiers having common gain profile with respect to a predetermined range of wavelengths, are coupled to the optical links for amplifying the optical signals. Multiple transmitters (24) emit multiple optical signals at predetermined different output values which are selected in accordance with the gain profile of amplifiers.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following:

(1) network compensation method;

(2) transmitter module; and

(3) pre-emphasis adjustment method.

USE - Optical communication network.

ADVANTAGE - As the optical signals are output with respect to gain profile of amplifiers, the gain variation due to amplifier profiles is minimized in up to four spans and the transmitters are set with correct operating power. Also the power fluctuation range at optical receivers in

DESCRIPTION OF DRAWING(S) - The figure shows the block diagram of the optical communication network.
transmitters 24
Dwg.3/7

L6 ANSWER 3 OF 11 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
Full Text
AN 2002-156065 [21] WPIDS
CR 2003-532493 [50]
DNN N2002-118695
TI Concentration measurement of component in a solution e.g. human blood, by determining ratio of two e.g. optical signals according to parameter that is assessed based on ratio of factors in order of two signals to cancel out noise.
DC P31 S02 S05 T01
IN WANG, G; WANG, W; WANG, G C; **WANG, W K**; WANG, J
PA (WANG-I) WANG W; (WANG-I) WANG W K; (WANG-I) WANG G
CYC 5
PI DE 10102346 A1 20010920 (200221)* 24
FR 2806480 A1 20010921 (200221)
GB 2361533 A 20011024 (200221)
US 2001023391 A1 20010920 (200221)
TW 542714 A 20030721 (200406)
US 2004210120 A1 20041021 (200470)
GB 2361533 B 20041110 (200474)
ADT DE 10102346 A1 DE 2001-10102346 20010119; FR 2806480 A1 FR 2001-768 20010119; GB 2361533 A GB 2001-1137 20010116; US 2001023391 A1 US 2001-766237 20010119; TW 542714 A TW 2000-104938 20000317; US 2004210120 A1 CIP of US 2001-766237 20010119, US 2004-752437 20040106; GB 2361533 B GB 2001-1137 20010116
PRAI TW 2000-104938 20000317
AB DE 10102346 A UPAB: 20041117
NOVELTY - Two signals undergo mathematical e.g. Fourier transformation and a parameter, KO, is assessed based on the ratio of the factor in the order of the first signal to the factor in the order of the second signal. The ratio of the two signals is determined from the assessed KO parameter, to cancel out the noise components of the two signals.
DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for a device for determining the concentration of a dissolved material in a solvent of a solution in a container.
USE - For measuring a dissolved material in a solvent e.g. in-vivo human blood component analysis in a human finger of e.g. glucose, oxyhaemoglobin, uric acid, cholesterol, or for e.g. immunological, enzymatic or drug action studies.
ADVANTAGE - Measures concentration of dissolved material in solvent exactly and effectively, by avoiding e.g. optical noise scatter.
DESCRIPTION OF DRAWING(S) - The figure shows a mechanical device used for measuring the concentration of a sample to be tested.
Dwg.1/2

L6 ANSWER 4 OF 11 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
Full Text
AN 2001-537557 [60] WPIDS
DNN N2001-399322
TI Pen having a correction fluid reservoir used for writing and correcting purposes comprises a tubular holder, a correction fluid container, an upper tubular element, a refill, and a cap.
DC P77
IN WANG, W; **WANG, W K**
PA (WANG-I) WANG W K; (WANG-I) WANG W
CYC 4
PI DE 20110650 U1 20010906 (200160)* 10
GB 2382328 A 20030528 (200343)#
CA 2364205 A1 20030603 (200348)# EN
FR 2833521 A3 20030620 (200348)#
ADT DE 20110650 U1 DE 2001-20110650 20010627; GB 2382328 A GB 2001-28202 20011126; CA 2364205 A1 CA 2001-2364205 20011203; FR 2833521 A3 FR 2001-16401 20011218
PRAI DE 2001-20110650 20010627; GB 2001-28202 20011126;
CA 2001-2364205 20011203; FR 2001-16401 20011218
AB DE 20110650 U UPAB: 20011018
NOVELTY - Pen having a correction fluid reservoir comprises a tubular holder (10) provided with an attachment (103) at the upper end having a circular inner flange and a circular recess (101) above the circular flange, a correction fluid container (13), whose lower end (13a) fits tightly into the circular recess and presses against the circular flange, and an upper tubular element (11) engaging with the attachment. A refill (12) fits into the tubular holder and its upper end runs through the circular flange and touches the lower end of the correction fluid container. A cap (14) engages with a lower end of the tubular holder and with an upper end of the upper tubular element.
USE - Used for writing and correcting purposes.

DESCRIPTION OF DRAWING(S) - The drawing shows an exploded view of the pen.
tubular holder 10
tubular element 11
refill 12
correction fluid container 13
lower end 13a
cap 14
circular recess 101
attachment 103
Dwg.1/5

L6 ANSWER 5 OF 11 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN

Full Text

AN 2000-375371 [32] WPIDS

DNN N2000-281929 DNC C2000-113279

TI Detection of charged particles in aqueous solution where electrodes are immersed by passing stepped potential and measuring impulse current.

DC B04 J04 P31 S05 X25

IN WANG, W K

PA (WANG-I) WANG W K

CYC 1

PI US 6061585 A 20000509 (200032)* 8

ADT US 6061585 A CIP of US 1995-563006 19951127, US 1997-901532 19970728

PRAI US 1997-901532 19970728; US 1995-563006 19951127

AB US 6061585 A UPAB: 20000706

NOVELTY - The charged particles are detected by immersing electrode (A) into the aqueous solution

to create a conductive path between the solution and the second electrode (B) which is not immersed in the solution A stepping potential is then applied to the electrodes and the impulse current through the solution is measured and compared with an impulse current of a reference solution. The aqueous solution can be within a biological body, e.g. a rat. The reference solution contains known ingredients one of which is lactate.

USE - To eliminate transient response to electrodes for electrocardiogram or electroencephalogram.

ADVANTAGE - Non given.

DESCRIPTION OF DRAWING(S) - The drawing shows a schematic diagram of the apparatus for detecting charged particles

Electrodes A,B

Dwg.4/5

L6 ANSWER 6 OF 11 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN

Full Text

AN 1984-239472 [39] WPIDS

CR 1981-46448D [26]

DNN N1984-179249 DNC C1984-101064

TI Fluorescence detection appts. for tagged biological particles - attached to others bound to reflective substrate.

DC B04 D16 J04 S03 S05

IN WANG, W K

PA (WANG-I) WANG W

CYC 1

PI GB 2136953 A 19840926 (198439)* 10

GB 2136953 B 19850320 (198512)

ADT GB 2136953 A GB 1979-20976 19790803; GB 2136953 B GB 1983-20976 19830803

PRAI GB 1979-20976 19790803; GB 1979-42836 19791212;

GB 1983-20976 19830803

AB GB 2136953 A UPAB: 19930925

The detection apparatus includes a stimulating optical beam which impinges the sample at a shallow angle. Perpendicular to the plane of the sample is a fluorescence detector downstream of two narrow band interference filters with similar peak wavelengths which attenuate strongly at the frequency of the stimulating beam. Excess photons from the stimulating beam are reflected from the substrate and enter a photon trap.

USE - The apparatus measures the degree of fluorescence from tagged samples esp. in antibody-antigen reactions. The sensitivity of the detection system is improved by the use of a reflecting substrate which reduces the number of stimulating photons which reach the detector.

3A/5

L6 ANSWER 7 OF 11 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN

Full Text

AN 1982-79273E [38] WPIDS

TI Copper refining anode slime processing - by ammonium acetate leaching to extract lead.

DC M25

IN CHUANG, W S; HOH, Y C; LEE, B D; MA, T; WANG, W K

PA (NUCL-N) INST NUCLEAR ENERGY

CYC 7

PI EP 59806 A 19820915 (198238)* EN 16

US 4352786 A 19821005 (198242)
JP 57149437 A 19820916 (198243)
EP 59806 B 19841107 (198445) EN
R: BE DE FR GB NL
DE 3167023 G 19841213 (198451)
JP 61003857 B 19860205 (198609)
ADT EP 59806 A EP 1981-301045 19810312; JP 57149437 A JP 1981-31064 19810304
PRAI EP 1981-301045 19810312
AB EP 59806 A UPAB: 19930915
Lead is removed from copper refining anode slime by (a) primary leaching with ammonium acetate soln. to remove at least 48% of the lead content of the slime; (b) secondary leaching of the residue with ammonium acetate soln. so that at least 94% of the original lead content of the slime is removed; (c) combining the two leach solns; and (d) concentrating the combined soln. to crystallise out lead acetate.
The method allows lead recovery from the slime and upgrades the slime to allow further recovery of selenium, tellurium, tin, silver, gold, etc.

L6 ANSWER 8 OF 11 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
Full Text
AN 1982-B5100E [06] WPIDS
TI Electrical safety socket with internal lock - enables electrical source to be connected at same time as plug is locked.
DC V04
IN WANG, W K
PA (WANG-I) WANG W
CYC 1
PI US 4312554 A 19820126 (198206)* 6
PRAI US 1980-116715 19800130
AB US 4312554 A UPAB: 19930915
The electric socket has locking units inside. When the plug is inserted into it, the electrical source will be connected, and at the same time, the plug will be locked. To disconnect the plug, a button must first be pushed to release the lock and disconnect the electrical source.
This construction will be very safe because there is no way to contact the electrical source from the socket either with the plug inserted or without the plug inserted. When it is connected, accidental disengagement of the plug and disconnection of the electrical source will not occur.
2

L6 ANSWER 9 OF 11 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
Full Text
AN 1981-79504D [43] WPIDS
TI Recovery of precious metals from decopperised anode slime - by leaching, extraction and recovery for improved selenium recovery rate.
DC M25
IN CHUANG, I S; HOH, Y C; WANG, W K
PA (NUCL-N) INST NUCLEAR ENERGY
CYC 1
PI US 4293332 A 19811006 (198143)* 7
PRAI US 1979-46685 19790608; US 1980-132493 19800320
AB US 4293332 A UPAB: 19930915
Decopperised anode slime is treated 3 times with 4-9M HNO3 for at least 40 mins. at 40-115 deg. C to produce leach soln. contg. (by wt.) 95% of the Ag, 96% of the Se, and 75% of the Te content. Denitrating and chlorinating is carried out by liq.-liq. extraction with 5-100 vol.% neutral or basic org. solvent balanced with hydrocarbon diluent and 8M or more HCl (acidity adjuster). Te is sepd. from Se, Pb or Cu by liq.-liq. extraction with 5-100 vol.% org. solvent balanced with org. extractant, 4M or more HCl as scrubbing soln. and 1M or less HCl as stripping soln. Se is pptd. from soln. with SO2 at 35.1-45 deg. C and a free acid content of 3.5-4.5M HCl. Te is pptd. from Te-contg. soln. with SO2 at 10-45 deg. C and a free acid content of more than 0.1M HCl. The HNO3 leach residue is treated with aqua regia at 40-110 deg. C for at least 1 hr. to produce residue contg. at least 99.46 wt.% of Au content. Au is sepd. by liq.-liq. extraction at below 30 deg. C with 10-12C ether and 2M (or higher) HCl scrubbing soln., and is recovered by treating with oxalic acid at 40-100 deg. C for at least 3 hrs.
The process is carried out at lower temps., saving energy. The Se recovery rate is much higher, being at least 94%, whereas previous recovery rates did not exceed 80%. Less off-gas is produced, thus reducing pollution.

L6 ANSWER 10 OF 11 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
Full Text
AN 1981-46448D [26] WPIDS
TI Solid-phase immunoassay - using substrate with metal surface.
DC B04 J04 S03
IN WANG, W K
PA (WANG-I) WEI-KUNG WANG
CYC 2

DE 3042535 A 19820211 (198207)
 DE 3025022 A 19820722 (198230)
 GB 2065298 B 19850220 (198508)
 DE 3025022 C 19850704 (198528)
 DE 3042535 C 19860821 (198634)
 ADT GB 2065298 A GB 1979-42836 19791212; DE 3042535 A DE 1980-3025022
 19800702; DE 3025022 A DE 1980-3042535 19800702
 PRAI GB 1979-42836 19791212
 AB GB 2065298 A UPAB: 19930915
 Detection of biological particles of a particular type (I) in a fluid
 comprises (a) immersing a substrate with a metal surface in a soln. contg.
 biological (protein) particles (II), other proteins and salts until a
 monomolecular protein layer has been adsorbed on the surface; (b) washing
 the substrate with water to remove unbound protein; (c) immersing the
 substrate in the test fluid so that (I), if present, is bound to (II); (d)
 binding fluorescent-labelled biological particles (III) to the immobilised
 (I); (e) washing with water to remove unbound particles; (f) irradiating
 the substrate with excitation light; and (g) directing the fluorescent
 light to a detector while directing the reflected excitation light away
 from the detector.
 Appts. comprises (a) a highly reflective substrate with a surface for
 receiving a sample; (b) a light source directing excitation light towards
 the sample; (c) a detector for measuring the fluorescence from the sample;
 and (d) means for directing reflected excitation light away from the
 detector.
 Method can be used to detect antibodies, hormones, specific- binding
 proteins, receptors or antigens in biological fluids.

L6 ANSWER 11 OF 11 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN

Full Text

AN 1981-04041D [04] WPIDS

TI Recovery of precious metals from anode slime - using hydrometallurgical
 method involving leaching, solvent, solvent extraction, PPTN. and redn.
 e.g. for recovering gold and silver.

DC E36 M25

IN CHUANG, W S; HOB, Y C; SHAW, I S; WANG, W K

PA (NUCL-N) INST NUCLEAR ENERGY

CYC 6

PI EP 20826 A 19810107 (198104)* EN

R: BE DE FR GB NL

JP 56003630 A 19810114 (198111)

EP 20826 B 19830720 (198330) EN

R: BE DE FR GB NL

DE 2965903 G 19830825 (198335)

JP 61003384 B 19860131 (198609)

JP 43003384 B (198612)

ADT JP 56003630 A JP 1979-75975 19790615

PRAI EP 1979-301148 19790614

AB EP 20826 A UPAB: 19930915

Gold, silver, selenium and tellurium are separated and recovered from
 anode slime by leaching, solvent extn., precipitation and reduction. The
 process comprises (a) nitric acid leaching of the slime, (b) silver
 chloride pptn., (c) simultaneous denitration and chlorination, (d) sepn.
 of Se and Te by liq.-liq. extraction, (e) stripping of Te, (f) redn. of Se
 and Te by sulphur dioxide, (g) aqua regia leaching, (h) sepn. of Au from
 Ag, platinumoids and other impurities by liq.-liq. extn., and (i) direct
 redn. of the Au values from the extractant..

The process is for recovery of precious metals from decopperised
 anode slimes.

The energy consumption of the process is much lower than for the
 pyrometallurgical methods previously used, and the materials cost is low
 since all of the organic media and most of the mineral acids can be
 recycled and reused. The amount of wastes is small and they are
 relatively simple to treat. Selenium recovery is higher than prior art and
 gold and silver are recovered without formation of an intermediate Au-Ag
 alloy.

=> d his

(FILE 'HOME' ENTERED AT 22:54:50 ON 03 FEB 2006)

FILE 'USPATFULL' ENTERED AT 22:55:09 ON 03 FEB 2006

E WANG WEI KUNG/IN

L1 12 S E3

L2 1163 S (DENGUE VIRUS)

L3 727 S L2 AND (PCR OR POLYMERASE CHAIN REACTION)

L4 178 S L3 AND (PRIMER?/CLM OR OLIGONUCLEOTIDE?/CLM)

L5 112 S L4 AND AY<2003

FILE 'WPIDS' ENTERED AT 22:59:34 ON 03 FEB 2006

E WANG WEI KUNG/IN

L6 11 S E3

=> s (dengue virus)
459 DENGUE
42219 VIRUS

L7 213 (DENGUE VIRUS)
(DENGUE(W)VIRUS)

=> s 17 and (PCR or polymerase chain reaction)
11191 PCR
11929 POLYMERASE
210978 CHAIN
455132 REACTION
6896 POLYMERASE CHAIN REACTION
(POLYMERASE(W)CHAIN(W)REACTION)

L8 14 L7 AND (PCR OR POLYMERASE CHAIN REACTION)

=> s 18 not 16

L9 14 L8 NOT L6

=> d 19,bib,ab,1-14

L9 ANSWER 1 OF 14 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
Full Text
AN 2006-077950 [08] WPIDS
DNN N2006-067659 DNC C2006-028018
TI Detecting presence of different target nucleic acids in sample,
simultaneously, by performing primer extension using sample and mass
tagged primers, cleaving tags from extended primers, determining presence
and sizes of cleaved mass tags.
DC B04 D16 J04 S03
IN BRIESE, T; JU, J; LIPKIN, W I
PA (BRIE-I) BRIESE T; (JUJJ-I) JU J; (LIPK-I) LIPKIN W I
CYC 1
PI US 2006003352 A1 20060105 (200608)* 81
ADT US 2006003352 A1 Provisional US 2004-566967P 20040429, US 2005-119231
20050428
PRAI US 2004-566967P 20040429; US 2005-119231 20050428
AB US2006003352 A UPAB: 20060201
NOVELTY - Detecting (M1) presence of different target nucleic acids in a
sample, simultaneously, involves contacting sample with several nucleic
acid primers for carrying out primer extension, where for each target
nucleic acid at least one specific primer having mass tag of predetermined
size is used, separating unextended primers from extended primers,
cleaving mass tags from extended primers, and determining presence and
sizes of cleaved mass tags.
DETAILED DESCRIPTION - Detecting (M1) the presence of one or more of
several different target nucleic acids, in a sample, simultaneously,
comprises:
(a) contacting the sample with several nucleic acid primers
simultaneously and under conditions permitting, and for a time sufficient
for, primer extension to occur, where:
(i) for each target nucleic acid at least one predetermined primer is
used which is specific for that target nucleic acid;
(ii) each primer has a mass tag of predetermined size bound to the
primer through a labile bond; and
(iii) the mass tag bound to any primer specific for one target
nucleic acid has a different mass than the mass tag bound to any primer
specific for any other target nucleic acid;
(b) separating any unextended primers from any extended primers;
(c) simultaneously cleaving the mass tags from any extended primers;
and
(d) simultaneously determining the presence and sizes of any mass
tags so cleaved, where the presence of a cleaved mass tag having the same
size as a mass tag of predetermined size previously bound to a
predetermined primer indicates the presence in the sample of the target
nucleic acid specifically recognized by that predetermined primer.
USE - (M1) is useful for simultaneously detecting the presence of one
or more of several different target nucleic acids in a sample, where at
least one target nucleic acid is from a pathogen. The pathogen is chosen
from Bacillus anthracis, **Dengue virus**, West Nile virus, Japanese
encephalitis virus, St. Louis encephalitis virus, Yellow Fever virus, La
Crosse virus, California encephalitis virus, Rift Valley Fever virus, CCHF
virus, VEE virus, EEE virus, WEE virus, Ebola virus, Marburg virus, LCMV,
Junin virus, Machupo virus, Variola virus, severe acute respiratory
syndrome (SARS) coronavirus, enterovirus, influenza virus, parainfluenza
virus, respiratory syncytial virus, bunyavirus, flavivirus and alpha
-virus. The pathogen is a respiratory pathogen chosen from respiratory
syncytial virus A, respiratory syncytial virus B, influenza A (N1),
influenza A (N2), influenza A (M), influenza A (H1), influenza A (H2),
influenza A (H3), influenza A (H5), influenza B, SARS coronavirus, 229E
coronavirus, OC43 coronavirus, metapneumovirus European, metapneumovirus

4A, Parainfluenza 4B, cytomegalovirus, measles virus, adenovirus, enterovirus, M. pneumoniae, L. pneumophila and C. pneumoniae. The pathogen is an encephalitis-inducing pathogen chosen from West Nile virus, St. Louis encephalitis virus, Herpes Simplex virus, HIV-1, HIV-2, Neisseria meningitidis, Streptococcus pneumoniae, Haemophilus influenzae, Influenza B, SARS coronavirus, 229E-CoV, OC43-CoV, cytomegalovirus and Varicella Zoster virus. The pathogen is a hemorrhagic fever-inducing pathogen. The sample is a forensic sample, food sample, or blood or its derivative. The sample is a biological warfare agent or suspected biological warfare agent (claimed).

(M1) is useful in differential diagnosis of infectious disease, blood product surveillance, forensic microbiology and biodefense.

ADVANTAGE - (M1) enables simultaneous detection of one or more of several different target nucleic acids in a sample, simultaneously. (M1) enables detection of target nucleic acids using PCR/mass spectrometry system, cost-effectively and rapidly with high sensitivity, where the advantageous of the system include:

(i) hybridization to only two sites (forward and reverse primer binding sites) is required when compared to real time PCR where an intermediate third oligonucleotide is used (probe binding site), thus enhances flexibility in primer design;

(ii) tried and proven consensus PCR primers can be used, thus reduces the time and resources need for obtaining new reagents and assay controls;

(iii) large repertoire of tags allow highly multiplexed assays, and

(iv) maintains sensitivity of real time PCR.

Dwg.0/23

L9 ANSWER 2 OF 14 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN

Full Text

AN 2006-048147 [05] WPIDS

DNC C2006-018069

TI Novel fused heterocyclic compound, or its salt or solvate, for pharmaceutical composition such as hepatitis-C-therapeutic agent, has anti-HCV effect.

DC B02

IN ABE, K; ENDOH, T; FUJISHITA, T; IWATA, M; MAKINO, I; MATSUMOTO, H; NAITO, A; ONODERA, N

PA (SHIO) SHIONOGI & CO LTD

CYC 111

PI WO 2005121132 A1 WO 20051222 (200605)* JA 294

RW: AT BE BG BW CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IS IT
KE LS LT LU MC MW MZ NA NL OA PL PT RO SD SE SI SK SL SZ TR TZ UG
ZM ZW

W: AE AG AL AM AT AU AZ BA BB BG BR BW BY BZ CA CH CN CO CR CU CZ DE
DK DM DZ EC EE EG ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG
KM KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NA NG NI
NO NZ OM PG PH PL PT RO RU SC SD SE SG SK SL SM SY TJ TM TN TR TT
TZ UA UG US UZ VC VN YU ZA ZM ZW

ADT WO 2005121132 A1 WO 2005-JP10548 20050609

PRAI JP 2005-117548 20050414; JP 2004-173757 20040611

AB WO2005121132 A UPAB: 20060120

NOVELTY - A novel fused heterocyclic compound (I, II or III), or its salt or solvate, is new.

DETAILED DESCRIPTION - A novel fused heterocyclic compound of formula (I, II or III), or its salt or solvate, is new.

A = N or CH;

Het = any one of group of formulae (a-d), preferably group of formula (a or c);

R0 = H, optionally substituted alkyl, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted aralkyl, optionally substituted heteroaralkyl, -COORa or -CONRbRc;

Ra = H or ester residue;

Rb,Rc = H or amido residue;

R = same as R0 excluding H;

R1 = H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted aralkyl, optionally substituted heteroaralkyl, optionally substituted heterocyclic group, optionally substituted aryl carbonyl, optionally substituted heteroaryl carbonyl, optionally substituted aryloxy, optionally substituted arylthio, optionally substituted arylsulfonyl or NRdRe;

Rd,Re = H or amino residue;

R2 = same as R1 including hydroxy, alkylthio or alkyl sulfonyl, preferably H;

R3 = optionally substituted alkyl, alkenyl, halogen, nitro, alkoxy or optionally substituted amino;

p = 0-3;

R4 = hydroxy, carboxy, halogen, alkylhalide, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, alkoxy, alkenyloxy, alkoxy carbonyl, nitro, nitroso, optionally substituted amino, azide, aryl, aralkyl, cyano,

alkyl sulfonyl, optionally substituted carbamoyl, optionally substituted sulfamoyl, acyl, formyloxy, halo formyl, oxal, thio formyl, thio carboxyl, dithio carboxy, thio carbamoyl, sulfin, sulfo, sulfo amino, hydrazine, azide, ureido, optionally substituted amidino, guanidine, phthalimide, oxo, optionally substituted heteroaryl, optionally substituted heterocyclic, halogenated alkoxy, hydroxyalkyl, carbamoyl oxy alkyl, optionally substituted alkoxy alkyl, optionally substituted aminoalkyl, optionally substituted carbamoyl, optionally substituted carbamoyl alkyloxy, alkyl sulfonyl oxy, optionally substituted carbamoyl oxy, optionally substituted carbamoyl carbonyl, or optionally substituted amino alkyl oxy; and

q = 0-5.

When Het is group of formula (a), then R0 is H, R is alkyl, R and R1 are alkyl, and R2 is optionally substituted heteroaralkyl. When Het is group of formula (b), then R0 is H, R exists in adjacent position of N, and is aryl or heteroaryl, R1 is aryl and R2 is alkyl. When p is 1-3, at least one of R3 is alkoxy substituted on carbon atom of benzene ring adjacent to carbon atom at which Het is substituted, and carbon atom on benzene ring at which A is substituted, and R is optionally substituted benzyl. An INDEPENDENT CLAIM is included for pharmaceutical composition containing the fused heterocyclic compound, or its salt or solvate.

ACTIVITY - Virucide.

MECHANISM OF ACTION - Inhibits replication of virus. The ability of fused heterocyclic compound to inhibit HCV virus replication was studied as follows. The HCV virus replicon cell was maintained in the Dulbecco modified Eagle culture medium (DMEM) containing fetal calf serum (FCS) (10%), modified eagle medium (MEM) nonessential-amino acid solution (1x), and G418 (30 µg/ml). The compound was diluted in DMEM containing a fetal calf serum (2%) and MEM nonessential-amino acid solution (1x) and dimethyl sulfoxide (1%). The replicon cell (2.5x10⁴ cells) was then cultured in fetal calf serum (FCS), in 5% CO₂ at 37 deg. C, for three days, in 96 well cell culture plate. A control was run simultaneously, without adding the test compound. All RNA was eluted with RNase free water (100 µl), and was collected by centrifugation (1200 rpm) for 5 minutes. Real-time PCR using GeneAmp 5700 sequence detection system, was performed, to measure the copy number of the HCV. Results showed IC₅₀ value of 0.1 µM or less.

USE - Used for pharmaceutical composition such as anti-hepatitis-C-virus (HCV) drug, hepatitis-C-virus agent, and hepatitis-C-therapeutic agent. The virus is yellow fever virus, **dengue virus**, West Nile virus or pesti virus.

ADVANTAGE - The fused heterocyclic compound provides pharmaceutical composition with excellent anti-HCV effect, direct inhibitory effect with respect to enzyme of virus, metabolism stability, CYP inhibition and solubility.

Dwg.0/0

L9 ANSWER 3 OF 14 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN

Full Text

AN 2005-747861 [76] WPIDS

DNC C2005-227884

TI Determining whether an agent preferentially binds to allelic variant of L-SIGN comprises separately contacting an agent with allelic variants of L-SIGN, cell lines and plasma membrane fractions from extracts of cell lines.

DC B04 D16

IN GARDNER, J P; OLSON, W C

PA (PROG-N) PROGENICS PHARM INC

CYC 109

PI WO 2005100601 A2 20051027 (200576)* EN 162

RW: AT BE BG BW CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IS IT
KE LS LT LU MC MW MZ NA NL OA PL PT RO SD SE SI SK SL SZ TR TZ UG
ZM ZW

W: AE AG AL AM AT AU AZ BA BB BG BR BW BY BZ CA CH CN CO CR CU CZ DE
DK DM DZ EC EE EG ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG
KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NA NI NO NZ
OM PG PH PL PT RO RU SC SD SE SG SK SL SM SY TJ TM TN TR TT TZ UA
UG US UZ VC VN YU ZA ZM ZW

ADT WO 2005100601 A2 WO 2005-US10381 20050325

PRAI US 2004-556725P 20040326

AB WO2005100601 A UPAB: 20051125

NOVELTY - Determining whether an agent preferentially binds to at least one allelic variant of liver/lymph node-specific ICAM-3-grabbing nonintegrin (L-SIGN) comprises separately contacting an agent with one of at least two allelic variants of L-SIGN, at least two cell lines expressing allelic variants of L-SIGN, and plasma membrane fractions from extracts of the at least two cell lines, under conditions suitable for binding of the agent.

DETAILED DESCRIPTION - The method comprises:

(A) separately contacting an agent with one of at least two allelic variants of L-SIGN, at least two cell lines expressing allelic variants of L-SIGN, and plasma membrane fractions from extracts of the at least two cell lines, under conditions suitable for binding of the agent; and

variants, or to the cells or membrane fractions expressing allelic variants with which the agent is contacted, where a difference in relative binding indicates that the agent preferentially binds to at least one allelic variant of L-SIGN.

INDEPENDENT CLAIMS are included for the following:

(1) a method for determining whether an agent preferentially binds to a first allelic variant of L-SIGN;

(2) a method for screening agents, not known to bind to any allelic variant of L-SIGN, to identify an agent that preferentially binds to at least one the allelic variant of L-SIGN;

(3) a method for identifying a monoclonal antibody that specifically binds to an allelic variant of L-SIGN;

(4) an agent that preferentially binds at least one allelic variant of L-SIGN;

(5) a composition comprising the agent, and a carrier;

(6) a method for treating a subject afflicted with a pathogen-related disorder, susceptibility to which is associated with at least one polymorphism in L-SIGN;

(7) a method for preventing infection of a subject by a pathogen, susceptibility to which infection is associated with at least one polymorphism in L-SIGN;

(8) a method for inhibiting in a subject the onset of a pathogen-related disorder, susceptibility to which is associated with at least one polymorphism in L-SIGN;

(9) a method for preventing infection of a subject by a pathogen, which infection is prevented by immunizing the subject;

(10) a method for inhibiting in a subject the onset of a pathogen-related disorder, the inhibition of which is effected by immunizing the subject;

(11) a method for predicting resistance of a subject to infection by a pathogen by determining the status of L-SIGN Exon 4 repeat polymorphisms in the subject and correlating that status to a degree of resistance of the subject to the pathogen;

(12) a method for predicting the susceptibility of a subject to infection by a pathogen by identifying single nucleotide L-SIGN polymorphisms in the subject and correlating the presence of the single nucleotide, polymorphisms (SNPs) to the susceptibility of the subject to the pathogen;

(13) an article of manufacture comprising a packaging material containing in it, the agent cited above and a label providing instructions for using the agent to treat a subject afflicted with a pathogen-associated disorder; and

(14) an article of manufacture comprising a packaging material containing in it one of an allelic L-SIGN protein variant substantially identical to an L-SIGN variant associated with membranes of cells of a subject, and an expression vector comprising a nucleic acid that encodes the allelic L-SIGN protein variant, and a label providing instructions for using the L-SIGN variant protein or expression vector to prevent infection of the subject by a pathogen, which infection is prevented by using the L-SIGN protein variant as an immunogen to immunize the subject.

ACTIVITY - Antiinflammatory; Hepatotropic; Virucide; Respiratory-Gen; Fungicide; Antibacterial; Protozoacide; Schistosomacide; Antimalarial.

MECHANISM OF ACTION - L-SIGN modulator; L-SIGN agonist.

USE - The method is useful for determining whether an agent preferentially binds to at least one allelic variant of L-SIGN (claimed).
Dwg.0/9

L9 ANSWER 4 OF 14 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN

Full Text

AN 2005-702118 [72] WPIDS

DNC C2005-213481

TI New system comprising a container comprising a nucleic acid amplification mix, a lateral flow test device comprising a reservoir area, a dye area and a test area, useful for detecting target nucleic acid.

DC B04 D16

IN CHOI, Y H; JUNG, J; KIM, Y

PA (ACCE-N) ACCESS BIO INC

CYC 1

PI US 2005227275 A1 20051013 (200572)* 40

ADT US 2005227275 A1 Provisional US 2004-560197P 20040407, Provisional US 2004-567845P 20040503, US 2005-102001 20050407

PRAI US 2005-102001 20050407; US 2004-560197P 20040407;
US 2004-567845P 20040503

AB US2005227275 A UPAB: 20051109

NOVELTY - A system for detecting target nucleic acid comprising a container, a lateral flow test device, a dye area, and a test area, is new.

DETAILED DESCRIPTION - A new system for detecting target nucleic acid comprises:

(a) a container comprising a nucleic acid amplification mix comprising a primer labeled with different haptens at its 5' and 3' ends, and optionally dNTP labeled with a hapten to form a nucleic acid complex;

reagent conditions suitable for binding of a specific binding partner with the nucleic acid complex;

(c) a dye area comprising a specific binding partner to the nucleic acid complex, where the specific binding partner is linked or conjugated to a reporter dye or another hapten; and

(d) a test area comprising a different specific binding partner specific to a different aspect of the nucleic acid complex.

An INDEPENDENT CLAIM is included for assaying for the presence of a target nucleic acid in a sample.

USE - The system is useful for detecting target nucleic acid.

DESCRIPTION OF DRAWING(S) - The figure shows an illustration of the system for detecting target nucleic acid. 5(A) shows the test device of the system before testing, comprising sample well at the bottom part and a result reading window at the middle. 5(B) shows the test results after assay. Two lines indicate a positive and one line a negative. The control line serves as an internal built-in control.

Dwg.5/31

L9 ANSWER 5 OF 14 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN

Full Text

AN 2005-272810 [28] WPIDS

CR 2004-562161 [54]

DNN N2005-224172 DNC C2005-085228

TI Novel isolated unique genomic sequence, useful for identifying unique oligonucleotide sequences and identifying biological organisms e.g. *Bacillus anthracis*, **Dengue virus** and *Vaccinia* in environmental samples.

DC B04 D16 S03

IN CAPUCO, J A; ELEY, G D; ROBINSON, D A; SCHAUDIES, P R; VOCKLEY, J G

PA (SCIT-N) SCI APPL INT CORP

CYC 106

PI WO 2005017488 A2 20050224 (200528)* EN 128

RW: AT BE BG BW CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IT KE
LS LU MC MW MZ NL OA PT RO SD SE SI SK SL SZ TR TZ UG ZM ZW

W: AE AG AL AM AT AU AZ BA BB BG BR BW BY BZ CA CH CN CO CR CU CZ DE
DK DM DZ EC EE EG ES FI GB GD GE GH GM HR HU ID IN IS JP KE KG KP
KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NA NI NO NZ OM
PG PH PL PT RO RU SC SD SE SG SK SL SY TJ TM TN TR TT TZ UA UG UZ
VC VN YU ZA ZM ZW

ADT WO 2005017488 A2 WO 2004-US2000 20040123

PRAI US 2003-441806P 20030123; US 2003-441745P 20030123

AB WO2005017488 A UPAB: 20050504

NOVELTY - An isolated unique genomic sequence (I) comprising an isolated nucleic acid sequence of any one of SEQ ID No. 1-1023, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for:

(1) an inferred unique genomic sequence (II) comprising an isolated nucleic acid sequence of any one of SEQ ID No. 1024-1029 or any one of SEQ ID No. 2072-3241;

(2) a target (III) comprising a unique oligonucleotide sequence of any one of SEQ ID No. 1030-2071;

(3) an array (IV) for detection of at least one biological entity, comprising unique oligonucleotide sequences bound to the array in predetermined locations, where the unique oligonucleotide sequence can hybridize to unique genomic sequences from the at least one biological entity; and

(4) identifying (M1) a biological organism in a sample comprising immobilizing unique oligonucleotide sequences in predetermined locations on an array, where the predetermined locations are associated with a known biological organism, applying a sample containing labeled nucleic acid sequences from the biological organism to the array, permitting the immobilized unique oligonucleotide sequences on the array to hybridize with complementary labeled nucleic acid sequences from the biological organism, and detecting the labeled nucleic acid sequences hybridized to the unique oligonucleotide sequences in predetermined locations on the array, where the location of the label identifies the biological organism, and the labeled nucleic acid sequences hybridized to the unique oligonucleotide sequences in predetermined locations on the array are termed unique genomic sequences.

USE - (I), (IV) or (M1) is useful for identifying a biological organism in a sample (e.g. environmental sample, clinical sample, biological sample and food sample). The sample comprises one or more biological entities. The biological entities are chosen from Acytota, prokaryotes, eukaryotes, Protista, Fungi, Plantae, Animalia and Monera. The biological entity is genetically engineered, and is preferably a pathogen chosen from *B.anthraxis*, **Dengue virus**, Ebola virus, Arbovirus, *F.tularensis*, *C.perfringens*, *E.coli*, *Vaccinia*, *Y.pestis* and *B.melitensis*. (III) is useful for identifying a biological organism. (I) or (II) is useful for identifying a unique oligonucleotide sequence (all claimed).

ADVANTAGE - (I) enables efficient identification of biological pathogens.

Dwg.0/8

Full Text
AN 2004-624790 [60] WPIDS
DNN N2004-494119 DNC C2004-224608
TI Sample processing tubule has three segments having reagents, each is isolated by breakable seal, expandable to receive volume of fluid expelled from another segment, and compressible to contain no fluid when so compressed.
DC B04 D16 S03
IN CHEN, L; CHEN, S; KOPCZYNSKI, K R; LEMIEUX, B; WANG, Z
PA (CHEN-I) CHEN L; (CHEN-I) CHEN S; (KOPC-I) KOPCZYNSKI K R; (LEMI-I) LEMIEUX B; (WANG-I) WANG Z; (IQUU-N) IQUUM INC
CYC 109
PI US 2004161788 A1 20040819 (200460)* 27
WO 2004080597 A2 20040923 (200462) EN
RW: AT BE BG BW CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IT KE
LS LU MC MW MZ NL OA PT RO SD SE SI SK SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BW BY BZ CA CH CN CO CR CU CZ DE
DK DM DZ EC EE EG ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG
KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NA NI NO NZ
OM PG PH PL PT RO RU SC SD SE SG SK SL SY TJ TM TN TR TT TZ UA UG
US UZ VC VN YU ZA ZM ZW
AU 2004220626 A1 20040923 (200562)
EP 1603674 A2 20051214 (200582) EN
R: AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LI LT LU LV
MC MK NL PT RO SE SI SK TR
ADT US 2004161788 A1 Provisional US 2003-445304P 20030205, US 2004-773775
20040205; WO 2004080597 A2 WO 2004-US3541 20040205; AU 2004220626 A1 AU
2004-220626 20040205; EP 1603674 A2 EP 2004-737303 20040205, WO
2004-US3541 20040205
FDT AU 2004220626 A1 Based on WO 2004080597; EP 1603674 A2 Based on WO
2004080597
PRAI US 2003-445304P 20030205; US 2004-773775 20040205
AB US2004161788 A UPAB: 20040920
NOVELTY - A sample processing tubule (10) (I), having at least three segments (16), each of which is defined by the tubule, fluidly isolated, at least in part by a breakable seal (14), expandable to receive a volume of fluid expelled from another segment, and compressible to contain substantially no fluid when compressed, is new. At least three segments, each contains at least one reagent.
DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for processing (M1) a sample, comprising:
(a) introducing a sample into a tubule discretized by breakable seals into several of fluidly isolated segments, where the tubule has a proximal end for receiving waste and a distal end for conducting an assay;
(b) incubating the sample in a segment of the tubule with a substance capable of specific binding to a preselected component of the sample;
(c) removing waste from the preselected component by clamping the tubule distally of the segment containing the preselected component and compressing that segment; and
(d) releasing a reagent to mix with the preselected component from a fluidly isolated adjacent distal segment by compressing at least one of the segment containing the preselected component and a segment containing a reagent distal of the segment, thereby opening a breakable seal and either propelling the reagent into the segment containing the preselected component or propelling the preselected component into the segment containing the reagent.
USE - (M1) is useful for processing a sample chosen from cells, bacteria, spores, virus, microbial organism, bucal cell, cervical cells, biopsy tissues, stool, biological fluid, allantic fluid, amniotic fluid, ascitic fluid, bile, bile acid, bile salts, bile pigment, blood, blood plasma, blood serum, cerebrospinal fluid, chorionic fluid, colostrums, digestive juice, gastric juice, intestinal juice, pancreatic juice, exudates, hemolymph, lochia, lymph, chyle, milk, mucus, pericardial fluid, peritoneal fluid, perspiration, pleural fluid, saliva, sebum, semen, seminal fluid, sputum, synovial fluid, tear, transdate, urine, vaginal fluid, soil, and environment water (all claimed).
ADVANTAGE - (I) is prepackaged with reagents for a desired sample processing protocol and thus provides the materials for an entire assay in one convenient package. Waste products can be easily segregated from a target of impressed early in the processing, and so the processed sample does not come into contact with surface that have been touched by the unprocessed sample. Only trace amounts of reaction inhibitors are present in the unprocessed sample that might coat the walls of the tubule are less likely to contaminate the processed sample.
DESCRIPTION OF DRAWING(S) - The drawing shows a front elevation view of a sample tube including a tubule.
Sample tube 1
Transparent flexible tubule 10
First opening 12
Breakable seals 14
Segment 16
Cap 20

Flexible membrane or septum 24
Sample collection tool 26
PCR reagent 290.
Dwg.1A/10

L9 ANSWER 7 OF 14 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN

Full Text

AN 2004-499087 [47] WPIDS

DNC C2004-184843

TI Detecting **Dengue virus** by a **polymerase chain reaction**, comprises providing a test sample of RNA suspected to be **Dengue virus** RNA, reverse transcribing a cDNA copy, amplifying target DNA, and detecting amplified products of target DNA.

DC B04 D16

IN CALLAHAN, J D; TEMENAK, J J

PA (CALL-I) CALLAHAN J D; (TEME-I) TEMENAK J J; (USNA) US SEC OF NAVY

CYC 1

PI US 2004126387 A1 20040701 (200447)* 12

US 6855521 B2 20050215 (200513)

ADT US 2004126387 A1 US 2000-726345 20001201; US 6855521 B2 Provisional US 1999-168184P 19991201, US 2000-726345 20001201

PRAI US 2000-726345 20001201; US 1999-168184P 19991201

AB US2004126387 A UPAB: 20040723

NOVELTY - Detecting (M1) the presence of **Dengue virus** by a **polymerase chain reaction (PCR)**, comprises providing **Dengue virus** RNA or a test sample of RNA suspected of being **Dengue virus** RNA, reverse transcriptase (RT) enzymes, dATPs, dGTPs, dCTPs, dTTPs and buffer, so that reverse transcription of a cDNA copy occurs, and detecting the presence of amplification products of a target sequence of DNA.

DETAILED DESCRIPTION - Detecting (M1) presence of **Dengue virus** by **PCR**, involves providing the RNA of the **Dengue virus** or a test sample of RNA suspected of being **Dengue virus** RNA, reverse transcriptase (RT) enzymes, dATPs, dGTPs, dCTPs, dTTPs and buffer containing divalent cations such as magnesium cation in sufficient quantities such that reverse transcription of a cDNA copy occurs, providing group specific or serotype-specific primers and probes of Dengue in sufficient quantities such that amplification of a target sequence of DNA occurs, and detecting the presence of the amplification products of the target sequence of DNA as an indication of the presence of **Dengue virus**.

INDEPENDENT CLAIMS are also included for the following:

(1) forward oligonucleotide primer for dengue-1, 2, 3 and 4 viruses, comprising a sequence of 5'-gacaccacaccttggacaa-3', 5'-ccgcgtgtcgactgtacaa-3', 5'-gggaaaaccgtctatcaata-3' and 5'-tgaagagattctcaaccggac-3', respectively;

(2) reverse oligonucleotide primer for dengue-1, 2, 3 and 4 viruses, comprising a sequence of 5'-cacctggctgtcacctccat-3', 5'-caggcccatgaacagttttaa-3', 5'-cgccataaccaatttcattgg-3' and 5'-aatccctgctgtgtgtggg-3', respectively;

(3) oligonucleotide probe for dengue-1, 2, 3 and 4 viruses, comprising a sequence of 5'-agagggtgtttaaagagaaagtgacacgcg-3', 5'-ttggaatgctgcaggggacgagga-3', 5'-cacagttggcgaagagattctcaacagga-3' and 5'-tcatacagttttgcgagtcctttcca-3', respectively;

(4) group specific forward oligonucleotide primer for **dengue virus**, comprising a sequence of 5'-aaggactagaggttakaggagaccc-3';

(5) group specific reverse oligonucleotide primer for **dengue virus**, comprising a sequence of 5'-ggcgytctgtgcctggawtgatg-3';

(6) group specific oligonucleotide probe for **dengue virus** serotypes 1 and 3, comprising a sequence of 5'-5-carboxyfluorescein (FAM)-aacagcatattgacgctgggagagacc-N,N,N',N'-tetramethyl-6-carboxyrhodamine (TAMRA)-3'; and

(7) group specific oligonucleotide probe for **dengue virus** serotypes 2 and 4, comprising a sequence of 5'-MAX-aacagcatattgacgctgggaaagacc-TAMRA-3'.

USE - (M1) is useful for detecting the presence of **Dengue virus** by **PCR**. (M1) is useful for detecting the presence of dengue-1, 2, 3 or 4 virus by **PCR**, which involves providing the RNA of the dengue-1, 2, 3 or 4 virus or a test sample of RNA suspected of being dengue-1, 2, 3 or 4 virus RNA, RT enzymes, dATPs, dGTPs, dCTPs, dTTPs and buffer containing divalent cations in sufficient quantities such that reverse transcription of cDNA copy occurs, providing serotype-specific primers and probe for dengue-1, 2, 3 or 4 virus for amplification of target sequence of DNA, and detecting the presence of the amplification products of the target sequence of DNA as an indication of the presence of dengue-1, 2, 3 or 4 virus (claimed). (M1) is useful for testing acute phase serum samples from patients clinically suspected to have dengue infection, where the diagnostic assay is useful in guiding clinical care during the acute phase of illness.

ADVANTAGE - (M1) enables qualitative detection of any dengue serotype in research samples. (M1) enables quantitative measurement of **dengue virus** in research samples. (M1) enables rapid and sensitive detection of **dengue virus** for the epidemiological study of dengue infections.

Dwg.0/1

L9 ANSWER 8 OF 14 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
Full Text
AN 2004-035140 [03] WPIDS
DNC C2004-011681
TI Identifying a virus strain in a sample comprises contacting a nucleic acid from the sample with at least one pair of oligonucleotide primer pairs that hybridize to the sequences of the nucleic acid.
DC B04 D16
IN ECKER, D J; GRIFFEY, R H; HOFSTADLER, S A; MCNEIL, J; SAMPATH, R
PA (ECKE-I) ECKER D J; (GRIF-I) GRIFFEY R H; (HOFI-I) HOFSTADLER S A; (MCNE-I) MCNEIL J; (SAMP-I) SAMPATH R; (ISIS-N) ISIS PHARM INC
CYC 103
PI WO 2003100035 A2 20031204 (200403)* EN 117
RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IT KE LS
LU MC MW MZ NL OA PT SD SE SI SK SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK
DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR
KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NI NO NZ OM PH PL
PT RO RU SC SD SE SG SK SL TJ TM TN TR TT TZ UA UG US UZ VC VN YU
ZA ZM ZW
US 2003228571 A1 20031211 (200410)
AU 2003269809 A1 20031212 (200443)
ADT WO 2003100035 A2 WO 2003-US9802 20030331; US 2003228571 A1 Provisional US 2002-369405P 20020401, US 2003-405756 20030331; AU 2003269809 A1 AU 2003-269809 20030331
FDT AU 2003269809 A1 Based on WO 2003100035
PRAI US 2002-369405P 20020401; US 2003-405756 20030331
AB WO2003100035 A UPAB: 20040112
NOVELTY - Identifying a virus strain in a sample comprises contacting a nucleic acid from the sample with at least one pair of oligonucleotide primer pairs that hybridize to the sequences of the nucleic acid, where the sequences flank a variable acid sequence of the virus strain.
DETAILED DESCRIPTION - Identifying a virus strain in a sample comprises:
(a) contacting a nucleic acid from the sample with at least one pair of oligonucleotide primer pairs that hybridize to the sequences of the nucleic acid, where the sequences flank a variable acid sequence of the virus strain;
(b) amplifying the variable nucleic acid sequence to produce an amplification product;
(c) determining the molecular mass or base composition of the amplification product; and
(d) identifying the virus strain.
An INDEPENDENT CLAIM is included for a method of distinguishing a first virus strain in a sample from at least a second virus strain by:
(a) contacting nucleic acid from the first virus strain in the sample with at least one pair of oligonucleotide primers which hybridize to sequences of the nucleic acid, wherein the sequences flank a variable acid sequence of the first virus strain;
(b) amplifying the variable nucleic acid sequence to produce an amplification product;
(c) determining the molecular mass or base composition of the amplification product; and
(d) distinguishing the first virus strain.
USE - The methods are useful for identifying virus strain in a sample (claimed).
Dwg.0/12

L9 ANSWER 9 OF 14 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
Full Text
AN 2003-229576 [22] WPIDS
DNC C2003-059120
TI New chimeric nucleic acids, useful in vaccines against dengue fever, encode fusion proteins that include fragments of dengue E protein and bacterial dehydrogenase.
DC B04 D16
IN CHINEA SANTIAGO, G; ESPINOSA PEREZ, R R; GUILLEN NIETO, G E; GUZMAN TIRADO, M G; HERMIDA CRUZ, L; LAZO VAZQUEZ, L; LOPEZ ABARRATEGUI, C; RODRIGUEZ DIAZ, R; SIERRA VAZQUEZ, B D L C; SILVA RODRIGUEZ, R D L C; VALDES PRADO, I; ZULUETA MORALES, A; ABARRATEGUI, C L; CRUZ, L H; DE LA C SILVA RODRIGUEZ, R; DE LA CARIDAD SIERRA VAZQUEZ BRI, PEDRO; DIAZ, R R; MORALES, A Z; NIETO, G E G; PEREZ, R R E; PRADO, I V; SANTIAGO, G C; TIRADO, M G G; VAZQUEZ, L L; DE LA CARIDAD SIERRA VAZQUEZ BPK, PEDRO; GUILLEN, N G E; HERMIDA, C L; SILVA, R R D L; VAZQUEZ, B D L C
PA (INGG-N) CENT ING GENETICA & BIOTECNOLOGIA; (MEDI-N) INST MEDICINA TROPICAL PEDROKOURI; (IPKM-N) IPK INST MEDICINA TROPICAL KOURI PEDRO; (MEDI-N) INST MEDICINA TROPICAL KOURI IPK PEDRO; (ABAR-I) ABARRATEGUI C L; (DIAZ-I) DIAZ R R; (GUIL-I) GUILLEN N G E; (HERM-I) HERMIDA C L; (MORA-I) MORALES A Z; (PERE-I) PEREZ R R E; (PRAD-I) PRADO I V; (SANT-I) SANTIAGO G C; (SILV-I) SILVA R R D L C; (TIRA-I) TIRADO M G G; (VAZQ-I) VAZQUEZ B D L C S; (VAZQ-I) VAZQUEZ L L
CYC 101

RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR IE IT KE LS LU
MC MW MZ NL OA PT SD SE SK SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK
DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR
KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT
RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG US UZ VN YU ZA ZM
ZW

EP 1418180 A2 20040512 (200431) EN
R: AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR IE IT LI LT LU LV MC
MK NL PT RO SE SI SK TR

AU 2002325784 A1 20030303 (200452)
KR 2004030056 A 20040408 (200453)
BR 2002011178 A 20040810 (200455)
US 2004234951 A1 20041125 (200478)
ZA 2004000289 A 20041124 (200481) 125
JP 2004537306 W 20041216 (200482) 580
CN 1531548 A 20040922 (200503)
MX 2004000486 A1 20050301 (200568)

ADT WO 2003008571 A2 WO 2002-CU6 20020712; EP 1418180 A2 EP 2002-760073
20020712, WO 2002-CU6 20020712; AU 2002325784 A1 AU 2002-325784 20020712;
KR 2004030056 A KR 2004-700610 20040115; BR 2002011178 A BR 2002-11178
20020712, WO 2002-CU6 20020712; US 2004234951 A1 WO 2002-CU6 20020712, US
2004-484114 20040607; ZA 2004000289 A ZA 2004-289 20040114; JP 2004537306
W WO 2002-CU6 20020712, JP 2003-514888 20020712; CN 1531548 A CN
2002-814350 20020712; MX 2004000486 A1 WO 2002-CU6 20020712, MX 2004-486
20040116

FDT EP 1418180 A2 Based on WO 2003008571; AU 2002325784 A1 Based on WO
2003008571; BR 2002011178 A Based on WO 2003008571; JP 2004537306 W Based
on WO 2003008571; MX 2004000486 A1 Based on WO 2003008571

PRAI CU 2001-172 20010716
AB WO2003008571 A UPAB: 20030402

NOVELTY - Chimeric nucleotide sequences (I) which are specific
combinations of sequences (S1) encoding parts of protein E of flavivirus
and sequences (S2) corresponding to a gene encoding a mutant MDH
(Neisseria meningitidis dehydrogenase), are new. (I) encode chimeric
proteins (II) that can induce a neutralizing and protective antibody
response to flavivirus. S2 is a 1851 (S3), 1821 (S4) or 168 (S5) base pair
sequence, all given in the specification.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for (II)
produced by expressing (I).

ACTIVITY - Virucide.

Rats were immunized intraperitoneally with 35 micro g of chimeric
protein PLL1 (comprising the 286-426 region of **dengue virus** serotype 2
E protein fused to the C-terminus of a sequence representing the first 45
amino acids of MDH), in Freund's adjuvant. After the fourth dose, blood
was analyzed for **dengue virus**-2 antibodies by enzyme-linked
immunosorbent assay. The titer varied from 32000 to 512000, compare less
than 100 for unvaccinated controls). The antibodies induced did not
recognize other serotypes of the virus.

MECHANISM OF ACTION - Vaccine.

USE - (I), and proteins encoded by them, are used to prevent or treat
dengue fever, and for diagnosis and serotyping of **dengue virus**.

ADVANTAGE - (I) generate an antibody response that is
serotype-specific (recognition of antibodies to heterologous serotypes may
be a cause of hemorrhagic forms of dengue fever). A single chimeric
protein may be protective against two serotypes, so just two proteins may
offer complete protection.

Dwg.0/13

L9 ANSWER 10 OF 14 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
Full Text
AN 2003-182625 [18] WPIDS
DNC C2003-048102
TI New **dengue virus**-specific primers, useful for reverse
transcriptase-**polymerase chain reaction** assays, particularly for
detecting or quantitating **dengue virus** in a sample.

DC B04 D16
IN WANG, W
PA (WANG-I) WANG W
CYC 1
PI US 2002155435 A1 20021024 (200318)* 6
ADT US 2002155435 A1 Provisional US 2001-272535P 20010301, US 2002-85944
20020228
PRAI US 2001-272535P 20010301; US 2002-85944 20020228
AB US2002155435 A UPAB: 20030317

NOVELTY - A nucleic acid comprising a sequence that includes:
(i) a **dengue virus**-specific primer that is 18 - 28 nucleotides in
length, and includes 18 consecutive nucleotides of a 28-mer (dna1); or
(ii) a second **dengue virus**-specific primer that is 18 - 28
nucleotides in length, and includes 18 consecutive nucleotides of another
28-mer (dna2), is new.

DETAILED DESCRIPTION - A new nucleic acid comprises:

length, and includes 18 consecutive nucleotides of a 28-mer (dna1); or
(ii) a second **dengue virus**-specific primer that is 18 - 28
nucleotides in length, and includes 18 consecutive nucleotides of another
28-mer (dna2).

INDEPENDENT CLAIMS are also included for the following:

(1) detecting **dengue virus**;
(2) quantitating **dengue virus**;
(3) a kit for detecting **dengue virus** comprising:
(i) a **dengue virus**-specific primer that is 18 - 28 nucleotides in
length, and includes 18 consecutive nucleotides of a 28-mer (dna1); and
(ii) a second **dengue virus**-specific primer that is 18 - 28
nucleotides in length, and includes 18 consecutive nucleotides of another
28-mer (dna2); and

(4) an isolated nucleic acid comprising a fragment of a dengue viral
genome or its DNA copy, where the fragment includes:

(i) a sequence complementary or identical to 18 consecutive
nucleotides of dna1;
(ii) a second sequence complementary or identical to 18 consecutive
nucleotides of dna2; and
(iii) a non-naturally occurring deletion or insertion, the deletion
or insertion occurring in a region of the fragment flanked by the first
and the second sequence.

5'-cccatctctcaiaatccctgctgttg-3' (dna1)

5'-aatatgctgaacgcgagagaaaccgcg-3' (dna2)

USE - The **dengue virus**-specific primers are useful in reverse
transcriptase-polymerase chain reaction assays, particularly for
detecting or quantitating **dengue virus** in a sample (claimed).
Dwg.0/0

L9 ANSWER 11 OF 14 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN

Full Text

AN 2002-017625 [02] WPIDS

DNC C2002-005103

TI Design and utilization of specific DNA sequences found in the terminal 3'
terminal non-coding region, as specific nucleic acid-based diagnostic
system for rapid and specific flavivirus identification and
quantification.

DC B04 D16

IN HOUNG, H; KANESA-THASAN, N; HOUNG, H H

PA (USSA) US ARMY MEDICAL RES & MATERIAL COMMAND

CYC 95

PI WO 2001079546 A2 20011025 (200202)* EN 50

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ
NL OA PT SD SE SL SZ TZ UG ZW

W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DE DK DM
DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC
LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE
SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW

AU 2001012172 A 20011030 (200219)

EP 1276898 A1 20030122 (200308) EN

R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT
RO SE SI

BR 2000017215 A 20040210 (200414)

US 6793488 B1 20040921 (200462)

ADT WO 2001079546 A2 WO 2000-US28961 20001019; AU 2001012172 A AU 2001-12172
20001019; EP 1276898 A1 EP 2000-973687 20001019, WO 2000-US28961 20001019;
BR 2000017215 A BR 2000-17215 20001019, WO 2000-US28961 20001019; US
6793488 B1 Provisional US 1999-129713P 19990416, Provisional US
1999-153685P 19990914, US 2000-551161 20000414

FDT AU 2001012172 A Based on WO 2001079546; EP 1276898 A1 Based on WO
2001079546; BR 2000017215 A Based on WO 2001079546

PRAI US 2000-551161 20000414; US 1999-129713P 19990416;

US 1999-153685P 19990914

AB WO 200179546 A UPAB: 20020109

NOVELTY - Isolated DNA segments having specific DNA sequences related to
sequences found in the terminal 3'-noncoding region of flavivirus, are
new.

DETAILED DESCRIPTION - Isolated DNA segments having specific DNA
sequences related to sequences found in the terminal 3'-noncoding region
of flavivirus, are new. The Serotype-specific Upstream Primers are
(I)-(VII), the Serotype-specific fluorescent probes (VIII)-(XII) and the
Serotype-specific down-stream primers are (XIII)-(XVI) are as follows:

(I) 5'-GAT-CAA-GCT-TACA-CCA-GGG-GAA-GCT-GTA-TCC-TGG-3' (I) DV1-1U;

(II) 5'-GAT-CAA-GCT-TAAG-GTG-AGA-TGA-AGC-TGT-AGT-CTC-3' (II) DV2-2U;

(III) 5'-GAT-CAA-GCT-TAGC-ACT-GAG-GGA-AGC-TGT-ACC-TCC-3' (III)

DV3-1U;

(IV) 5'-GAT-CAA-GCT-TAAG-CCA-GGA-GGA-AGC-TGT-ACT-CCT-3' (IV) DV4-1U;

(V) 5'-CAAGCCCCCTCGAAGCTGT-3' (V) JE;

(VI) 5'-CAAGCCCCCTCGAAGCTGT-3' (VI) JE-U1(F214);

(VII) 5'-CCTGGGATAGACTAGGAGATCTTCTG-3' (VII) WNV-U1;

(VIII) 5'-CTG-TCT-CTA-CAG-CAT-CAT-TCC-AGG-CA-3' (VIII) DV1-P1;

(IX) 5'-CTG-TCT-CTG-CAA-CAT-CAA-TCC-AGG-CA-3' (IX) DV4-P1;

(XI) 5'-TCTGCTCTATCTCAACATCAGCTACTAGGCACAGA-3' (XI) JE.P1;
 (XII) 5'TCTGCACAACCAGCCACACGGC-3' (XII) WNV-P1;
 (XIII) 5'-CAA-TCC-ATC-TTG-CGG-CGC-TCT-3' (XIII) DV4-1L;
 (XIV) 5'-GAT-CGA-ATT-CCAT-TCC-ATT-TTC-TGG-CGT-TCT-3' (XIV) DV2-1L;
 (XV) 5'- CACCAGCTACATACTTCGGCG-3' (XV) JE.R382; and
 (XVI) 5'-CCATTGTCGGCGCACTG-3' (XVI) WNV-L1.

INDEPENDENT CLAIMS are also included for the following:

- (1) a **polymerase chain reaction (PCR)**-based diagnostic kit for detecting or quantitating flavivirus comprising isolated DNA segments having any one of the sequences (I-XVI) or complementary sequences;
- (2) a method (M1) for detecting or quantifying one or more species of flavivirus contained in a sample, comprising:
 - (i) collecting a sample suspected of containing a flavivirus;
 - (ii) preparing the sample for **PCR** amplification;
 - (iii) adding **PCR** reagents where the primer pairs are selected from sequences (I-XVI);
 - (iv) maintaining the sample under conditions suitable for amplification;
 - (v) detecting or quantifying one or more of the flavivirus species;
- (3) a method (M2) for detecting or quantifying **Dengue virus** comprising the steps of contacting a sample suspected of containing a flavivirus with **PCR** reagents, including at least two **PCR** primers selected from serotype upstream primers sequences (I-VIII) and down-stream primers sequences (XIII-XVI), polymerase enzyme and an oligonucleotide probe selected from serotype-specific fluorescent probe sequences (IX-XII) (the fluorescer molecule is attached to a first end of the oligonucleotide and a quencher molecule is attached to a second end so that the quencher quenches the fluorescer molecule whenever the oligonucleotide probe is in a single-stranded state and such that the fluorscer is substantially unquenched whenever the oligonucleotide probe is hybridized to the target nucleic acid. The 5' end is impervious to digestion by the 5' to 3' exonuclease activity or a polymerase, and the 3' end is impervious to the 5' to 3' extension activity of the polymerase. All contents are subjected to thermal cycling, including a polymerization step); and
- (4) a nucleic acid amplification assay (M3) and kit for detecting flavivirus which contains an improvement comprising a probe or primer having at least 15 contiguous nucleotides selected from the last 250 nucleotides of the 3' non-coding region of the flavivirus genome.

USE - The invention provides a specific rapid diagnosis of flaviviral infection which can be employed in a portable field-tested diagnostic kit in endemic regions. Four distinct **dengue virus** types (dengue 1,2,3 and 4) are each capable of causing infection in humans of which there has been a huge increase in transmission with the increasing incidence of dengue hemorrhagic fever.

ADVANTAGE - Prior art methods for flavivirus use serological detection which is time consuming and has limited sensitivity for detecting low levels of dengue virus. Use of rapid molecular diagnostic systems such as RT-PCR would provide a faster means of dengue virus identification from clinical samples. Also the probes of this invention are highly discriminatory such that even single nucleotide substitutions can be reliably detected among different viruses.
 Dwg.0/11

L9 ANSWER 12 OF 14 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN

Full Text

AN 2000-072885 [06] WPIDS

CR 2003-058572 [05]

DNC C2000-020998

TI Novel nucleic acid for use in vaccines.

DC B04 D16

IN CHANG, G J

PA (USSH) US DEPT HEALTH & HUMAN SERVICES; (USSH) US CENTERS DISEASE CONTROL & PREVENTION; (CHAN-I) CHANG G J

CYC 86

PI WO 9963095 A1 19991209 (200006)* EN 58

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL
 OA PT SD SE SL SZ UG ZW

W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB
 GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU
 LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR
 TT UA UG US UZ VN YU ZA ZW

AU 9943296 A 19991220 (200021)

BR 9910830 A 20010213 (200114)

EP 1084252 A1 20010321 (200117) EN

R: DE FR GB NL

JP 2002517200 W 20020618 (200242) 63

US 2003022849 A1 20030130 (200311)

AU 778988 B2 20041223 (200510)

ADT WO 9963095 A1 WO 1999-US12298 19990603; AU 9943296 A AU 1999-43296
 19990603; BR 9910830 A BR 1999-10830 19990603, WO 1999-US12298 19990603;
 EP 1084252 A1 EP 1999-955295 19990603, WO 1999-US12298 19990603; JP
 2002517200 W WO 1999-US12298 19990603, JP 2000-552289 19990603; US

19990603, US 2001-826115 20010404, CIP of US 2001-701536 20010618; AU 778988 B2 AU 1999-43296 19990603
FDT AU 9943296 A Based on WO 9963095; BR 9910830 A Based on WO 9963095; EP 1084252 A1 Based on WO 9963095; JP 2002517200 W Based on WO 9963095; AU 778988 B2 Previous Publ. AU 9943296, Based on WO 9963095
PRAI US 1998-87908P 19980604; US 2001-826115 20010404;
US 2001-701536 20010618
AB WO 9963095 A UPAB: 20050928
NOVELTY - Nucleic acid molecule (I) comprises a transcription unit (TU) for an immunogenic flavivirus antigen (Ag). When incorporated into a host cell, TU directs synthesis of Ag.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

- (1) host cells containing (I); and
- (2) vaccines containing (I) plus a carrier.

ACTIVITY - Antiviral.

MECHANISM OF ACTION - Vaccine.

USE - (I) are used in vaccines to protect against flavivirus infection. Also (not claimed) (I) can be used to produce Ag for analytical or diagnostic applications. Plasmid pCBE1-14 contains a fragment of nucleic acid encoding the pre-M and E proteins of Japanese encephalitis virus (JEV) cloned into pCBamp. It was administered intramuscularly (50-100 µg) to 3-day old mice. After 7 weeks all animals were seropositive for JEV and all were protected against subsequent challenge by the mouse-adapted SA14 strain of JEV (contrast 40% survival for animals inoculated with the commercial vaccine JE-VAX).

ADVANTAGE - (I) makes possible inexpensive and safe production of a storage-stable vaccine that has minimal risk of causing adverse immunological reactions to impurities. The vaccines elicit neutralizing antibodies and protective immunity very effectively (i.e. 100% protection), and since they contain only part of the viral genome they can not cause infection in those manufacturing or receiving them. The immunity conferred by the vaccine is transmitted to offspring through the milk.

Dwg.0/8

L9 ANSWER 13 OF 14 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN

Full Text

AN 1999-034671 [03] WPIDS

DNC C1999-010426

TI Detection of **dengue virus** infection - comprises use of reverse transcriptase-**polymerase chain reaction** as a rapid assay for all serological types.

DC B04 D16

IN ENNIS, F A; ISHIKO, H; SUDIRO, T M

PA (UYMA-N) UNIV MASSACHUSETTS

CYC 23

PI WO 9849351 A1 19981105 (199903)* EN 27

RW: AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE

W: AU CA JP KR

AU 9872621 A 19981124 (199914)

US 5939254 A 19990817 (199939)

ADT WO 9849351 A1 WO 1998-US8526 19980428; AU 9872621 A AU 1998-72621 19980428; US 5939254 A US 1997-840344 19970428

FDT AU 9872621 A Based on WO 9849351

PRAI US 1997-840344 19970428

AB WO 9849351 A UPAB: 19990122

The following are claimed: (1) a method of detecting **dengue virus** in a biological sample comprises: (a) incubating RNA extracted from the sample with reverse transcriptase and a first **dengue virus** specific primer (P1) under conditions sufficient for double stranded nucleic acid formation; (b) adding a second **dengue virus** specific primer (P2) and a thermostable DNA polymerase; (c) incubating under conditions sufficient to allow the double stranded nucleic acid, if present, to be **PCR** amplified and form reaction products, and (d) detecting the reaction products as an indication of **dengue virus** in the sample where P1 comprises 15-28 nucleotides, including at least 15 nucleotides of the sequence 5'-TCTCTCCAGCGTCAATA-3' (I) and is fully complementary to a region in the dengue viral nucleic acid complementary to sequence (I), and P2 comprises 15-28 nucleotides, including at least 15 nucleotides of the sequence 5'-AAACCGTGCTGCTGTAG-3' (II) and is fully complementary to a region in the dengue viral nucleic acid complementary to sequence (II). Alternatively, in step (b), the second primer is identical to a region in the dengue viral nucleic acid that includes sequence (II), and a third primer (P3) being 15-28 nucleotides, including at least 15 consecutive nucleotides of the sequence 5'-AACTGTGCAGCCTGTAG-3' (III) is added; (2) a kit comprising P1, P2 and optionally P3, and reagents for performing **RT-PCR**; (3) a method of quantitating **dengue virus** in a biological sample, comprising: (a) mixing RNA extracted from the sample with a known quantity of competitor RNA; (b) incubating the mixture with P1 under conditions sufficient for double stranded nucleic acid formation; (c) adding a second **dengue virus**-specific primer (P4) and a thermostable

double stranded nucleic acid to be **PCR** amplified and form reaction products; (e) detecting the reaction products, and (f) comparing the amount of reaction product obtained with the amount obtained in the absence of competitor RNA, or quantitating the reaction products obtained by comparison to known amounts of competitor RNA where P4 comprises 15-28 nucleotides, including at least 15 nucleotides of the sequence 5'-AAACCGTGCAGCCTGTAG-3' (IV) and is fully complementary to a region in the dengue viral nucleic acid complementary to sequence (IV); (4) a method of determining the serotype of **dengue virus** in a biological sample, comprising: (a) as in (1), but using labelled nucleotides in the **PCR** to form labelled reaction products; (b) adding aliquots of reaction products to separate microwells, each of which is coated with a probe specific for one of the four **dengue virus** serotypes, and (c) detecting hybridisation; (5) P1, P2, P3 and P4, and (6) sequences (I)-(IV).

USE - The diagnostic test is particularly useful to clinically identify children with **dengue virus**, allowing early management of patients with the infection (disclosed).

ADVANTAGE - The methods provide a means to test for all four **dengue virus** serotypes in a shorter time than prior art methods.

Dwg.0/2

L9 ANSWER 14 OF 14 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
Full Text
AN 1995-032125 [05] WPIDS
DNC C1995-014311
TI Detecting dengue and other flaviviruses with consensus primers - for
PCR amplification, also specific primers for individual dengue types.
DC B04 D16
IN CHAN, Y C; CHOW, V T; SEAH, C L; CHAN, Y; CHEONG, C Y; KHENG, C S L;
KWONG, V C T
PA (UYSI-N) UNIV SINGAPORE NAT
CYC 2
PI GB 2279652 A 19950111 (199505)* 12
GB 2279652 B 19971210 (199801)
SG 96150 A1 20030523 (200347)
ADT GB 2279652 A GB 1994-13185 19940630; GB 2279652 B GB 1994-13185 19940630;
SG 96150 A1 SG 1995-666 19940730
PRAI GB 1993-13718 19930702
AB GB 2279652 A UPAB: 19950404
Presence of dengue, or other flaviviruses, is detected by using consensus primers DVCU and DVCD in a **PCR** amplification of target fragments of biological or clinical samples contg. cDNA templates, synthesised from RNA of any of the 4 dengue viruses or other related flaviviruses. Also new are pairs of primers comprising DSP1-4 in conjunction with DVCU or DVCD, and diagnostic kits contg. them as sequencing primers for flavivirus-related DNA fragments within the N53 region of the flavivirus genome. (Primer sequences are given in specification).
USE - The consensus primers detect flaviviruses in general; DSP1-4 identify specific **dengue virus** types. Flaviviruses other than dengue that can be detected include Japanese encephalitis, Kunjin and yellow fever. The primers can also be used for epidemiological studies and vector surveillance.
ADVANTAGE - The primers provide quicker and more sensitive diagnosis than known cell cultures or immunological assays. Depending on the **dengue virus** type, assay sensitivity is 103-105 copies of viral RNA.
Dwg.0/0

=> file medline

COST IN U.S. DOLLARS	SINCE FILE	TOTAL
	ENTRY	SESSION
FULL ESTIMATED COST	114.71	496.93

FILE 'MEDLINE' ENTERED AT 23:01:45 ON 03 FEB 2006

FILE LAST UPDATED: 3 FEB 2006 (20060203/UP). FILE COVERS 1950 TO DATE.

On December 11, 2005, the 2006 MeSH terms were loaded.

The MEDLINE reload for 2006 will soon be available. For details on the 2005 reload, enter HELP RLOAD at an arrow prompt (=>).
See also:

<http://www.nlm.nih.gov/mesh/>
http://www.nlm.nih.gov/pubs/techbull/nd04/nd04_mesh.html
http://www.nlm.nih.gov/pubs/techbull/nd05/nd05_med_data_changes.html
http://www.nlm.nih.gov/pubs/techbull/nd05/nd05_2006_MeSH.html

OLDMEDLINE is covered back to 1950.

MEDLINE thesauri in the /CN, /CT, and /MN fields incorporate the MeSH 2006 vocabulary.

=> e wang w k/au

E1 2 WANG W I/AU
E2 148 WANG W J/AU
E3 60 --> WANG W K/AU
E4 124 WANG W L/AU
E5 2 WANG W L L/AU
E6 55 WANG W M/AU
E7 26 WANG W N/AU
E8 59 WANG W P/AU
E9 38 WANG W Q/AU
E10 8 WANG W R/AU
E11 73 WANG W S/AU
E12 25 WANG W T/AU

=> s e3

L10 60 "WANG W K"/AU

=> s l10 and dengue

4987 DENGUE

L11 1 L10 AND DENGUE

=> d l11,cbib,ab

L11 ANSWER 1 OF 1 MEDLINE on STN

2001038342. PubMed ID: 10970375. Quantitative competitive reverse transcription-PCR for quantification of **dengue** virus RNA. **Wang W K**; Lee C N; Kao C L; Lin Y L; King C C. (Institute of Microbiology, Taipei, Taiwan.. wwang60@yahoo.com) . Journal of clinical microbiology, (2000 Sep) 38 (9) 3306-10. Journal code: 7505564. ISSN: 0095-1137. Pub. country: United States. Language: English.

AB A quantitative competitive reverse transcription-PCR assay was developed to quantify **dengue** virus RNA in this study. The main features include a primer pair targeting a highly conserved region in the capsid and the addition of competing RNA that contains an internal deletion to provide a stringent internal control for quantification. It can be utilized to quantify RNA isolated from the four **dengue** virus serotypes but not RNA isolated from other flaviviruses, including Japanese encephalitis virus and hepatitis C virus, both prevalent in Asia. It can also be used to quantify **dengue** virus RNA isolated from the plasma of infected individuals. The sensitivity of the assay was estimated to be 10 to 50 copies of RNA per reaction, and twofold differences in virus titer are distinguishable. This assay is a convenient, sensitive, and accurate method for quantification and can be used to further understanding of the pathogenesis of **dengue** virus infection.

=> s (dengue virus)

4987 DENGUE

406963 VIRUS

L12 2828 (DENGUE VIRUS)

(DENGUE(W)VIRUS)

=> s l12 and (PCR or polymerase chain reaction)

143536 PCR

268290 POLYMERASE

394031 CHAIN

572588 REACTION

220565 POLYMERASE CHAIN REACTION

(POLYMERASE(W)CHAIN(W)REACTION)

L13 329 L12 AND (PCR OR POLYMERASE CHAIN REACTION)

=> s l13 and (primer? or oligonucleotide?)

103427 PRIMER?

1 OLIOGNUCLEOTIDE?

L14 78 L13 AND (PRIMER? OR OLIOGNUCLEOTIDE?)

=> s l14 and py<2003

13953642 PY<2003

L15 65 L14 AND PY<2003

=> d l15,cbib,ab,1-65

L15 ANSWER 1 OF 65 MEDLINE on STN

2004643474. PubMed ID: 15619909. The cloning and sequence analysis of cDNA of the partial E genomic region of Dengue type 4 virus from a Chinese patient. Li G; Yao J; Peng W; Wang F. (Department of Infectious Diseases, Sun Yatsen University of Medical Sciences, Guangzhou 510630.) Zhonghua shi yan he lin chuang bing du xue za zhi = Zhonghua shiyan he linchuang bingduxue zazhi = Chinese journal of experimental and clinical virology, (1997 Mar) 11 (1) 62-5. Journal code: 9602873. ISSN: 1003-9279. Pub.

AB The partial envelope (E) protein gene of **Dengue virus** type 4 (DV4), derived from the serum of a patient with dengue fever in an epidemic during October 1993 in Guangdong Province of China, was cloned and sequenced. DV RNA was converted to cDNA by reverse transcription with random **primers** before the **polymerase chain reaction** using DV4 specific **primers** was performed. The amplified product of 421bp was subsequently filled in 3' recessed ends, isolated, purified and inserted into pUC18 and pUC19 plasmid vector. Their sequences were determined by dideoxy nucleotide chain termination method. A comparison between DV4 Chinese strain and several other previously reported strains shows the homology to be 93.72% for the same dengue serotype, 61.26-64.40% for the other dengue serotypes, only 40.31% for Japanese encephalitis virus, which belongs to the member of flavivirus family. A conserved sequence with 12 amino acids observed from deduced protein sequence probably represents an essential functional element among flavivirus family.

L15 ANSWER 2 OF 65 MEDLINE on STN

2004593486. PubMed ID: 15566833. Semi-nested **PCR** using NS3 **primers** for the detection and typing of dengue viruses in clinical serum specimens. Seah C L; Chow V T; Chan Y C. (Department of Microbiology, Faculty of Medicine, National University of Singapore, Kent Ridge, 0511, Singapore.) Clinical and diagnostic virology, (1995 Aug) 4 (2) 113-20. Journal code: 9309653. ISSN: 0928-0197. Pub. country: Netherlands. Language: English.

AB BACKGROUND: More rapid, specific and sensitive tests for the laboratory diagnosis of **dengue virus** infections are needed. OBJECTIVE: To develop a semi-nested **polymerase chain reaction (PCR)** assay based on **primers** within the NS3 gene for the simultaneous detection and typing of dengue viruses in human sera. STUDY DESIGN: A first round of single-step reverse transcription-**polymerase chain reaction (RT-PCR)** was carried out with a pair of consensus **primers**, followed by a second round of semi-nested amplification using the upstream consensus **primer** and four type-specific down-stream **primers**. The sensitivity and specificity of the semi-nested **PCR** assay were determined using plaque- or TCID(50)-titrated virus-infected tissue culture fluid, and total RNA extracted from C6/36 cells infected with dengue and other flaviviruses, respectively. A retrospective study was performed on acute sera from thirteen patients with dengue (confirmed by virus isolation) employing semi-nested **PCR** in parallel with virus re-isolation and a single-step RT-**PCR** method for the typing of dengue viruses in human sera. RESULTS: The semi-nested **PCR** assay could detect up to 1 pfu of **dengue virus**, but not other flaviviruses. The semi-nested **PCR** and single-step RT-**PCR** assays correctly typed dengue viruses in twelve and five sera, respectively, whereas none of the sera was positive by virus re-isolation. CONCLUSIONS: This semi-nested **PCR** assay is a sensitive and specific tool for the detection and typing of dengue viruses from viremic human sera.

L15 ANSWER 3 OF 65 MEDLINE on STN

2002709426. PubMed ID: 12471426. Complete nucleotide sequence analysis of a Brazilian **dengue virus** type 2 strain. dos Santos Flavia Barreto; Miagostovich Marize Pereira; Nogueira Rita Maria Ribeiro; Edgil Dianna; Schatzmayr Hermann Goncalves; Riley Lee W; Harris Eva. (Departamento de Virologia, Instituto Oswaldo Cruz, Rio de Janeiro, RJ, Brasil.. Flaviab2001@aol.com) . Memorias do Instituto Oswaldo Cruz, (2002 Oct) 97 (7) 991-5. Journal code: 7502619. ISSN: 0074-0276. Pub. country: Brazil. Language: English.

AB In the last decade, dengue fever (DF) in Brazil has been recognized as an important public health problem, and an increasing number of dengue haemorrhagic fever (DHF) cases have been reported since the introduction of **dengue virus** type 2 (DEN-2) into the country in 1990. In order to analyze the complete genome sequence of a DEN-2 Brazilian strain (BR64022/98), we designed **primers** to amplify contiguous segments of approximately 500 base pairs across the entire sequence of the viral genome. Twenty fragments amplified by reverse transcriptase-**PCR** were cloned, and the complete nucleotide and the deduced amino acid sequences were determined. This constitutes the first complete genetic characterization of a DEN-2 strain from Brazil. All amino acid changes differentiating strains related to the Asian/American-Asian genotype were observed in BR64022/98, indicating the Asiatic origin of the strain.

L15 ANSWER 4 OF 65 MEDLINE on STN

2002692939. PubMed ID: 12454138. Detection of **dengue virus** replication in peripheral blood mononuclear cells from **dengue virus** type 2-infected patients by a reverse transcription-real-time **PCR** assay. Wang Wei-Kung; Sung Tzu-Ling; Tsai Yu-Chen; Kao Chuan-Liang; Chang Shu-Mei; King Chwan-Chuen. (Institute of Microbiology, College of Medicine, National Taiwan University, Taipei, Taiwan.. wwang60@yahoo.com) . Journal of clinical microbiology, (2002 Dec) 40 (12) 4472-8. Journal code: 7505564. ISSN: 0095-1137. Pub. country: United States. Language: English.

AB While **dengue virus** is thought to replicate in mononuclear phagocytic cells in vivo, attempts to detect it in peripheral blood mononuclear cells

generally low rates. In this study, we developed a reverse transcription (RT)-real-time **PCR** assay to quantify positive- and negative-sense RNA of **dengue virus** type 2 within the cells. The assay includes an RT step using either sense or antisense **primer** followed by a real-time **PCR** step using the designed **primers** and probe, which target a capsid region highly conserved in **dengue virus** type 2 strains. It can be used to monitor the dynamic change of intracellular **dengue virus** RNA species during the course of infection. When this assay is employed in quantification of **dengue virus** RNA species in PBMC from 10 patients infected with **dengue virus** type 2, both positive- and negative-sense dengue RNA can be detected, indicating that **dengue virus** is actively replicating in PBMC in vivo. Moreover, the amounts of negative-sense **dengue virus** RNA in PBMC correlate very well with the viral load of **dengue virus** in plasma, suggesting that quantification of negative-sense **dengue virus** RNA in PBMC may provide another indicator of **dengue virus** replication in vivo. Use of this convenient, sensitive, and accurate method of quantification in clinical samples from patients with different disease severity would further our understanding of the pathogenesis of dengue.

L15 ANSWER 5 OF 65 MEDLINE on STN

2002386885. PubMed ID: 12135294. Etiology of encephalitis syndrome among hospitalized children and adults in Takeo, Cambodia, 1999-2000. Srey Viseth Horm; Sadones Helene; Ong Sivuth; Mam Mony; Yim Chantham; Sor Sokhom; Grosjean Pierre; Reynes Jean-Marc; Grosjean Pierre; Reynes Jean-Marc. (Institut Pasteur du Cambodge, Phnom Penh, Cambodia.) American journal of tropical medicine and hygiene, (2002 Feb) 66 (2) 200-7. Journal code: 0370507. ISSN: 0002-9637. Pub. country: United States. Language: English.

AB Whether or not Japanese encephalitis virus (JEV) is an important causative agent of acute encephalitis in Cambodia remains unclear. This study was carried out to determine the cause of encephalitis syndrome among children and adults admitted to Takeo Provincial Hospital from October 1999 to September 2000. Ninety-nine cases were included in the study: 52 pediatric cases (12 were fatal) and 47 adult cases (10 were fatal). A causative agent such as human herpesvirus (HHV-3 or HHV-4), Cryptococcus neoformans, or Mycobacterium tuberculosis had been identified in 8 of the 11 adults who had human immunodeficiency virus type 1 (HIV-1). An infectious agent was identified in 35 (40%) of 88 HIV-1-seronegative patients (60% of the causes remains unidentified). These comprised 11 bacterial infections, 1 fungal infection, and 23 viral infections. The viral infections were 1 fatal HHV-4 infection, 5 **dengue virus** infections (2 fatal), 1 coinfection with flavivirus and alphavirus, and 16 presumptive infections JEV (no virus detected), one case of which was fatal. Infection with JEV, the principal cause identified in the 99 encephalitis syndromes, concerned 16 (31%) of 52 children.

L15 ANSWER 6 OF 65 MEDLINE on STN

2002384124. PubMed ID: 12132320. Phylogenetic analysis of the envelope protein (domain III) of dengue 4 viruses. Mota Javier; Ramos-Castaneda Jose; Rico-Hesse Rebeca; Ramos Celso. (Departamento de Arbovirus, Centro de Investigacion sobre Enfermedades Infecciosas, Instituto Nacional de Salud Publica, Cuernavaca, Morelos, Mexico.) Salud p'ublica de M'exico, (2002 May-Jun) 44 (3) 228-36. Journal code: 0404371. ISSN: 0036-3634. Pub. country: Mexico. Language: English.

AB OBJECTIVE: To evaluate the genetic variability of domain III of envelope (E) protein and to estimate phylogenetic relationships of dengue 4 (Den-4) viruses isolated in Mexico and from other endemic areas of the world. MATERIAL AND METHODS: A phylogenetic study of domain III of envelope (E) protein of Den-4 viruses was conducted in 1998 using virus strains from Mexico and other parts of the world, isolated in different years. Specific **primers** were used to amplify by RT-PCR the domain III and to obtain nucleotide sequence. Based on nucleotide and deduced aminoacid sequence, genetic variability was estimated and a phylogenetic tree was generated. To make an easy genetic analysis of domain III region, a Restriction Fragment Length Polymorphism (RFLP) assay was performed, using six restriction enzymes. RESULTS: Study results demonstrate that nucleotide and aminoacid sequence analysis of domain III are similar to those reported from the complete E protein gene. Based on the RFLP analysis of domain III using the restriction enzymes Nla III, Dde I and Cfo I, Den-4 viruses included in this study were clustered into genotypes 1 and 2 previously reported. CONCLUSIONS: Study results suggest that domain III may be used as a genetic marker for phylogenetic and molecular epidemiology studies of dengue viruses. The English version of this paper is available too at: <http://www.insp.mx/salud/index.html>.

L15 ANSWER 7 OF 65 MEDLINE on STN

2002373416. PubMed ID: 12118459. Predominance of the DEN-3 genotype during the recent dengue outbreak in Bangladesh. Aziz M M; Hasan K N; Hasanat M A; Siddiqui M A; Salimullah M; Chowdhury A K; Ahmed Moslehuddin; Alam M N; Hassan M S. (Department of Immunology, Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders BIRDEM,

- (2002 Mar) 33 (1) 42-8. Journal code: 0266303. ISSN: 0125-1562. Pub. country: Thailand. Language: English.
- AB A recent outbreak of dengue in Bangladesh was marked by many fatal complications. As clinical virulence varies among the genotypes of **dengue virus**, a study was conducted to investigate the molecular genotypes of dengue in Bangladesh. Reverse transcription **polymerase chain reaction** was used to determine viral genotypes using oligonucleotide generic **primers** that produce a 511 bp product. The resulting product was typed by nested **PCR** with strain-specific **primers**, yielding 482 (DEN-1), 119 (DEN-2), 290 (DEN-3) and 392 (DEN-4), visualized on UV transilluminator after electrophoresis on 2% agarose gel stained with ethidium bromide. Of 45 clinically diagnosed dengue patients (mean age 28 years; male/female 30/15), 19 (42.2%) had detectable viral RNA in their blood. However, during the first 5 days of fever in 30 patients, the frequency was 60% (18/30), implying that the sooner serum is drawn after the fever, the greater the chances of detecting viral RNA. DEN-3 was detected in all except 2 patients who were infected with DEN-2. DEN-2 (two cases) and DEN-4 (one case) were present as co-infections with DEN-3. All of the patients presented with fever, anorexia and vomiting; many had headache and general body ache; a few had a rash. About a quarter had suffered episodes of bleeding, while ascites, pleural effusion and CNS symptoms were found in a few patients. Patients positive for viral RNA were also positive for anti-dengue IgM ($p=0.007$) in subsequent sampling. The study suggests the predominance of DEN-3 infection with occasional co-infection with other types, during the recent outbreak of dengue in Bangladesh.
- L15 ANSWER 8 OF 65 MEDLINE on STN
2002346128. PubMed ID: 12089242. Rapid detection and quantification of RNA of Ebola and Marburg viruses, Lassa virus, Crimean-Congo hemorrhagic fever virus, Rift Valley fever virus, **dengue virus**, and yellow fever virus by real-time reverse transcription-**PCR**. Drosten Christian; Gottig Stephan; Schilling Stefan; Asper Marcel; Panning Marcus; Schmitz Herbert; Gunther Stephan. (Bernhard-Nocht-Institute of Tropical Medicine, Hamburg, Germany.. drosten@bni.uni-hamburg.de) . Journal of clinical microbiology, (2002 Jul) 40 (7) 2323-30. Journal code: 7505564. ISSN: 0095-1137. Pub. country: United States. Language: English.
- AB Viral hemorrhagic fevers (VHFs) are acute infections with high case fatality rates. Important VHF agents are Ebola and Marburg viruses (MBGV/EBOV), Lassa virus (LASV), Crimean-Congo hemorrhagic fever virus (CCHFV), Rift Valley fever virus (RVFV), **dengue virus** (DENV), and yellow fever virus (YFV). VHFs are clinically difficult to diagnose and to distinguish; a rapid and reliable laboratory diagnosis is required in suspected cases. We have established six one-step, real-time reverse transcription-**PCR** assays for these pathogens based on the Superscript reverse transcriptase-Platinum Taq polymerase enzyme mixture. Novel **primers** and/or 5'-nuclease detection probes were designed for RVFV, DENV, YFV, and CCHFV by using the latest DNA database entries. **PCR** products were detected in real time on a LightCycler instrument by using 5'-nuclease technology (RVFV, DENV, and YFV) or SybrGreen dye intercalation (MBGV/EBOV, LASV, and CCHFV). The inhibitory effect of SybrGreen on reverse transcription was overcome by initial immobilization of the dye in the reaction capillaries. Universal cycling conditions for SybrGreen and 5'-nuclease probe detection were established. Thus, up to three assays could be performed in parallel, facilitating rapid testing for several pathogens. All assays were thoroughly optimized and validated in terms of analytical sensitivity by using in vitro-transcribed RNA. The $\geq 95\%$ detection limits as determined by probit regression analysis ranged from 1,545 to 2,835 viral genome equivalents/ml of serum (8.6 to 16 RNA copies per assay). The suitability of the assays was exemplified by detection and quantification of viral RNA in serum samples of VHF patients.
- L15 ANSWER 9 OF 65 MEDLINE on STN
2002217332. PubMed ID: 11952948. **Dengue virus** infection rate in field populations of female *Aedes aegypti* and *Aedes albopictus* in Singapore. Chung Youne Kow; Pang Fung Yin. (Vector Control & Research Department, Ministry of the Environment, Singapore.. chung_youne_kow@env.gov.sg) . Tropical medicine & international health : TM & IH, (2002 Apr) 7 (4) 322-30. Journal code: 9610576. ISSN: 1360-2276. Pub. country: England: United Kingdom. Language: English.
- AB We developed a single-step reverse transcription-**polymerase chain reaction** (RT-**PCR**) followed by a semi-nested **PCR** using an upstream consensus **primer** and four type-specific **primers** within the non-structural protein gene (NS3) of dengue viruses to type dengue viruses in field populations of female *Aedes* mosquitoes. This yielded diagnostic fragments of 169, 362, 265 and 426 base pairs for **dengue virus** types 1, 2, 3 and 4, respectively. From 1997 to 2000, 54 (6.9%) of 781 *Aedes aegypti* and 67 (2.9%) of 2256 *Aedes albopictus* screened were positive for dengue viruses, with a declining trend. The most common **dengue virus** type detected in the *Aedes* mosquitoes was dengue-1. Details on the change of one serotype to another in the mosquito population over three

L15 ANSWER 10 OF 65 MEDLINE on STN

2002141780. PubMed ID: 11857532. Single rapid TaqMan fluorogenic probe based **PCR** assay that detects all four dengue serotypes. Warrilow David; Northill Judith A; Pyke Alyssa; Smith Greg A. (Queensland Health Scientific Services, Queensland, Australia.. David.Warrilow@health.qld.gov.au) . Journal of medical virology, (2002 Apr) 66 (4) 524-8. Journal code: 7705876. ISSN: 0146-6615. Pub. country: United States. Language: English.

AB Public health laboratories require rapid diagnosis of dengue outbreaks for application of measures such as vector control. We have developed a rapid single fluorogenic probe-based **polymerase chain reaction** assay for the detection of all four dengue serotypes (FUDRT-**PCR**). The method employs **primers** and probe that are complementary to the evolutionarily conserved 3' untranslated region of the dengue genome. The assay detected viral RNA of strains of all four dengue serotypes but not of the flaviviruses Japanese encephalitis virus, Murray Valley encephalitis virus, Kunjin, Stratford, West Nile, Alfuy or Yellow fever. When compared to an existing nested-**PCR** assay for the detection of dengue on clinical samples, FUDRT-**PCR** detected dengue 1 (100%, n=14), dengue 2 (85%, n=13), dengue 3 (64%, n=14) and dengue 4 (100%, n=3) with the indicated sensitivities. FUDRT-**PCR** enables diagnosis of acute dengue infection in four hours from sample receipt. In addition, a single-test procedure should result in a reduction in the number of tests performed with considerable cost savings for diagnostic laboratories.
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L15 ANSWER 11 OF 65 MEDLINE on STN

2001674223. PubMed ID: 11719982. A study of dengue imported to Kuwait during 1997-1999. Mustaf A S; Elbishbishi E A; Grover S; Pacsa A S; Al-Enezi A A; Chaturvedi U C. (Department of Microbiology, Faculty of Medicine, Kuwait University, Safat.. abusalin@hsc.kuniv.edu.kw) . Acta virologica. English ed, (2001 Apr) 45 (2) 125-8. Journal code: 0370401. ISSN: 0001-723X. Pub. country: Slovakia. Language: English.

AB This study was carried out on sera from 210 patients in Kuwait in 1997-1999. All of the patients were suffering from febrile illness and had recently visited dengue- (DEN) endemic areas. The sera were screened for DEN virus by inoculation into cultures of the Aedes albopictus cell clone C6/36 (virus isolation) and by IgM capture ELISA (detection of DEN virus-specific IgM antibodies). In the cell cultures, DEN virus could not be isolated from any of the patients' sera. However, sera from 19 patients were positive for DEN virus-specific IgM antibodies. All these 19 sera were tested for the presence of DEN virus-specific RNA by reverse transcription-**PCR** (RT-**PCR**) using DEN virus types-common (consensus) **primers**. In addition, the type of DEN virus was identified by using type-specific **primers** in a semi-nested **PCR**. The results showed that two of the 19 patients were infected with DEN virus type 2. This report of 19 patients with serological evidence of DEN infection indicates that imported DEN is a real threat to Kuwait, a country non-endemic for DEN but with a large portion of the population vacationing in DEN-hyperendemic areas during the peak DEN season and then returning to Kuwait.

L15 ANSWER 12 OF 65 MEDLINE on STN

2001574875. PubMed ID: 11682539. Development and evaluation of serotype- and group-specific fluorogenic reverse transcriptase **PCR** (TaqMan) assays for **dengue virus**. Callahan J D; Wu S J; Dion-Schultz A; Mangold B E; Peruski L F; Watts D M; Porter K R; Murphy G R; Suharyono W; King C C; Hayes C G; Temenak J J. (Viral and Rickettsial Diseases Department, Naval Medical Research Center, Silver Spring, Maryland 20910-7500, USA.) Journal of clinical microbiology, (2001 Nov) 39 (11) 4119-24. Journal code: 7505564. ISSN: 0095-1137. Pub. country: United States. Language: English.

AB Five fluorogenic probe hydrolysis (TaqMan) reverse transcriptase **PCR** (RT-**PCR**) assays were developed for serotypes 1 to 4 and group-specific detection of **dengue virus**. Serotype- and group-specific oligonucleotide **primers** and fluorogenic probes were designed against conserved regions of the **dengue virus** genome. The RT-**PCR** assay is a rapid single-tube method consisting of a 30-min RT step linked to a 45-cycle **PCR** at 95 and 60 degrees C that generates a fluorogenic signal in positive samples. Assays were initially evaluated against cell culture-derived dengue stock viruses and then with 67 dengue viremic human sera received from Peru, Indonesia, and Taiwan. The TaqMan assays were compared to virus isolation using C6/36 cells followed by an immunofluorescence assay using serotype-specific monoclonal antibodies. Viral titers in sera were determined by plaque assay in Vero cells. The serotype-specific TaqMan RT-**PCR** assay detected 62 of 67 confirmed **dengue virus**-positive samples, for a sensitivity of 92.5%, while the group-specific assay detected 66 of 67 confirmed **dengue virus**-positive samples, for a sensitivity of 98.5%. The TaqMan RT-**PCR** assays have a specificity of 100% based on the serotype concordance of all assays compared to cell culture isolation and negative results obtained when 21 normal human sera and plasma samples were tested. Our results demonstrate

sensitive, and specific screening and serotyping tools for epidemiological studies of **dengue virus** infections.

L15 ANSWER 13 OF 65 MEDLINE on STN

2001428270. PubMed ID: 11476326. Detection of dengue viruses in field caught male *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in Singapore by type-specific **PCR**. Kow C Y; Koon L L; Yin P F. (Vector Control and Research Department, Ministry of the Environment, Singapore.) Journal of medical entomology, (2001 Jul) 38 (4) 475-9. Journal code: 0375400. ISSN: 0022-2585. Pub. country: United States. Language: English.

AB Field male *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) adults caught from fixed monitoring stations weekly for 1 yr were screened for dengue viruses (DEN-1, DEN-2, DEN-3, and DEN-4). The assay was carried out using a single-step reverse transcription (or transcriptase)-**polymerase chain reaction (PCR)** (RT-PCR) followed by a semi-nested **PCR** using an upstream consensus **primer** and four type-specific **primers** within the nonstructural protein three gene (NS3) of dengue viruses. The diagnostic fragments for DEN-1, DEN-2, DEN-3, and DEN-4 serotypes were of sizes 169, 362, 265, and 426 bp, respectively. Results showed that in Singapore 1.33% and 2.15% of *Aedes aegypti* and *Aedes albopictus* adult male mosquitoes, respectively, were positive for dengue viruses. The serotypes detected in male *Ae. aegypti* was DEN-1 (44%), followed by DEN-2 (22.2%) and DEN-3 (22.2%), and DEN-4 (11.1%). For *Aedes albopictus* males, the serotype was DEN-4 (38.9%), followed by DEN-2 (33.3%), DEN-3 (16.7%), and DEN-1 (11.1%).

L15 ANSWER 14 OF 65 MEDLINE on STN

2001351182. PubMed ID: 11414440. Highly conserved nucleotide sequence and its deduced amino acids of the 5'-noncoding region and the capsid protein of a Bangkok isolate dengue-3 virus. Attatippaholkun W H; Attatippaholkun M K; Nisalak A; Vaughn D W; Innis B L. (Department of Clinical Chemistry, Faculty of Medical Technology, Mahidol University, Bangkok, Thailand.) Southeast Asian journal of tropical medicine and public health, (2000) 31 Suppl 1 119-25. Journal code: 0266303. ISSN: 0125-1562. Pub. country: Thailand. Language: English.

AB The dengue-3 virus genome encodes an uninterrupted open reading frame (ORF) flanked by 5' and 3' non-coding regions. The order of proteins encoded in dengue-3 virus ORF, as with other flaviviruses, is: Cap 5'-C-prM-E-NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5-3'. The nucleotide sequence of the 5'-noncoding region and the capsid protein of dengue-3 virus (a Bangkok isolate: CH53489 isolated by USAMC-AFRIMS in 1973) has been analyzed in both forward and reverse directions. The **PCR**-based cycle sequencing technique by the enzymatic method of Sanger et al (1977) using a sequencing **primer** 5'-end labeled with gamma32P-ATP is the method of our choice for sequencing analysis. One cDNA template was prepared by RT-**PCR** technique starting from the 5'-end nucleotide 1-465 of the dengue-3 genome. In our cycle sequencing experiments, the substitution of 7-deaza-dG was used for dG in DNA eliminated much of the secondary structures that produced gel artifacts. The final sequence result of this cDNA template was established from its sequence data determined on both strands in opposite directions. Alignment between the newly established nucleotide sequence as well as its deduced amino acid sequence of the Bangkok dengue-3 virus and the published sequence data of the dengue-3 prototype (H87) was manipulated by the PC-DOS-GIBIO-DNASIS TM 06-00 (Hitachi Software). According to the deduced amino acid sequence of the Bangkok dengue-3 virus, its C protein was found to be highly positively charged because of large numbers of lysine and arginine. The homology of the nucleotide sequence between the two dengue-3 virus revealed 97%. The deduced amino acid sequences from the nucleotides 95-465 of the two viruses showed the same indicating highly conserved capsid proteins. Multiple alignment of the nucleotide sequences as well as the deduced amino acid sequences among the Bangkok dengue-3 virus and other dengue 3 viruses also confirmed the highly conserved 5'-noncoding regions and the capsid proteins.

L15 ANSWER 15 OF 65 MEDLINE on STN

2001297807. PubMed ID: 11377710. Development of a fluorogenic RT-**PCR** system for quantitative identification of **dengue virus** serotypes 1-4 using conserved and serotype-specific 3' noncoding sequences. Houngh H S; Chung-Ming Chen R; Vaughn D W; Kanesa-thasan N. (Department of Virus Diseases, Walter Reed Army Institute of Research, Silver Spring, MD 20910, USA.) Journal of virological methods, (2001 Jun) 95 (1-2) 19-32. Journal code: 8005839. ISSN: 0166-0934. Pub. country: Netherlands. Language: English.

AB A fluorogenic reverse transcriptase-**polymerase chain reaction** (RT-**PCR**) system was developed for use as a rapid diagnostic test for determining dengue viremia. The **dengue virus** 3'-noncoding sequence was utilized to formulate serotype-specific RT-**PCR** assays for quantitative identification of the four different **dengue virus** serotypes. A generic RT **primer** set containing two dengue specific anti-sense **primers** (DV-L1 and DV-L2) could be used to transcribe extracted viral RNA of all four **dengue virus** types to complimentary

identified at the serotype level by the 5'-3' exonuclease assay using four serotype-specific sense **primers**. The fluorogenic dengue type-specific RT-PCR can detect each of the four dengue types at similar low detection limits, i.e. 20-50 plaque forming units per milliliter of serum. Two panels with four dengue reference serotypes and 134 clinical samples were used to validate detection sensitivity and specificity of the dengue serotype RT-PCR assay, using virus isolation in cell culture as the criterion standard. By analyzing sera samples from Puerto Rico that were collected from 1999 through 2000, the assay demonstrated high level detection sensitivity and specificity of 92.8 and 92.4%, respectively, for all four **dengue virus** serotypes.

L15 ANSWER 16 OF 65 MEDLINE on STN

2001038342. PubMed ID: 10970375. Quantitative competitive reverse transcription-PCR for quantification of **dengue virus** RNA. Wang W K; Lee C N; Kao C L; Lin Y L; King C C. (Institute of Microbiology, Taipei, Taiwan.. wwang60@yahoo.com) . Journal of clinical microbiology, (2000 Sep) 38 (9) 3306-10. Journal code: 7505564. ISSN: 0095-1137. Pub. country: United States. Language: English.

AB A quantitative competitive reverse transcription-PCR assay was developed to quantify **dengue virus** RNA in this study. The main features include a **primer** pair targeting a highly conserved region in the capsid and the addition of competing RNA that contains an internal deletion to provide a stringent internal control for quantification. It can be utilized to quantify RNA isolated from the four **dengue virus** serotypes but not RNA isolated from other flaviviruses, including Japanese encephalitis virus and hepatitis C virus, both prevalent in Asia. It can also be used to quantify **dengue virus** RNA isolated from the plasma of infected individuals. The sensitivity of the assay was estimated to be 10 to 50 copies of RNA per reaction, and twofold differences in virus titer are distinguishable. This assay is a convenient, sensitive, and accurate method for quantification and can be used to further understanding of the pathogenesis of **dengue virus** infection.

L15 ANSWER 17 OF 65 MEDLINE on STN

2000337422. PubMed ID: 10879258. Cellular proteins bind to the 3' and 5' untranslated regions of dengue 2 virus genome. Ong C C; Lam S K; AbuBakar S. (Department of Medical Microbiology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia.) Malaysian journal of pathology, (1998 Jun) 20 (1) 11-7. Journal code: 8101177. ISSN: 0126-8635. Pub. country: Malaysia. Language: English.

AB In vitro generated cloned full length dengue 2 virus untranslated regions (UTRs) were used in RNA gel mobility shift assays to examine cellular factors binding to the virus genomes. Cellular factors in lysates of Vero (monkey) and C6/36 (mosquito) cells bound specifically and non-specifically to the dengue 2 virus 3' UTR. Non-specific interaction with the 5' UTR, resulting in formation of at least 4 band shift complexes was noted with lysate of the C6/36 cells only. Pre-treating the cell lysates with proteinase K affected binding of cellular factors to the dengue 2 virus UTRs, suggesting that the cellular factors were proteins. These findings suggest that cellular proteins could interact with specific sites on the **dengue virus** genomes.

L15 ANSWER 18 OF 65 MEDLINE on STN

2000179949. PubMed ID: 10713370. Quantitative detection of dengue 2 virus using fluorogenic RT-PCR based on 3'-noncoding sequence. Houn H H; Hritz D; Kanasa-thasan N. (Department of Enteric Infections and Virus Diseases, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, DC 20307, USA.) Journal of virological methods, (2000 Apr) 86 (1) 1-11. Journal code: 8005839. ISSN: 0166-0934. Pub. country: Netherlands. Language: English.

AB A fluorescent DNA probe (DV2.P1) specific to the conserved distal 3'-noncoding region (nucleotides 10653-10678) of dengue 2 virus and a pair of flanking **primers** (DV2.L1 and DV2.U2) were designed to formulate a dengue 2-specific fluorogenic **polymerase chain reaction (PCR)**. In addition, DV2.L1 was also used as a reverse transcription (RT) **primer** to generate superior cDNA from dengue viral RNA. Optimal assay conditions with zero background were established to detect low levels of dengue 2 virus from clinical specimens. The range of **dengue virus** detection in spiked human sera was determined to be from 10 to 10(6) infectious virions per milliliter (plaque forming units determined using Vero cell line). Dengue 2 virus isolates from different geographic regions can be detected universally and identified by the fluorogenic RT-PCR assay. Moreover, the assay is specific for dengue 2 virus and does not recognize other related flaviviruses, including dengue serotypes 1, 3 and 4, Japanese encephalitis, St. Louis encephalitis, yellow fever, and Kunjin viruses. The assay also detected efficiently immunocomplexed dengue viruses. In practice, the fluorogenic RT-PCR assay detected readily viremia in sera collected from individuals ill with dengue fever. The rise and fall of dengue 2 virus concentrations in rhesus monkeys, reflecting viral proliferation and clearance, was also clearly illustrated by the assay.

2000164720. PubMed ID: 10699044. Rapid subtyping of **dengue virus** serotypes 1 and 4 by restriction site-specific **PCR**. Miagostovich M P; dos Santos F B; Gutierrez C M; Riley L W; Harris E. (Department of Virology, Oswaldo Cruz Institute, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil.) Journal of clinical microbiology, (2000 Mar) 38 (3) 1286-9. Journal code: 7505564. ISSN: 0095-1137. Pub. country: United States. Language: English.

AB We previously reported a simple subtyping method, restriction site-specific **PCR** (RSS-**PCR**), for **dengue virus** serotypes 2 and 3; here we describe its application for subtyping **dengue virus** serotypes 1 and 4. Three major RSS-**PCR** types were observed for **dengue virus** serotype 1 and two types were observed for **dengue virus** serotype 4, in agreement with previous strain classifications based on sequence analysis. Because of its simplicity, this method is amenable to rapid subtyping and application to epidemiological studies of dengue in countries where dengue is endemic.

L15 ANSWER 20 OF 65 MEDLINE on STN

2000137149. PubMed ID: 10674684. Detection and genetic relationship of **dengue virus** sequences in seventeen-year-old paraffin-embedded samples from Cuba. Sariol C A; Pelegrino J L; Martinez A; Arteaga E; Kouri G; Guzman M G. (Department of Virology, Pan American Health Organization/World Health Organization Collaborating Center for Viral Diseases, Havana, Cuba.) American journal of tropical medicine and hygiene, (1999 Dec) 61 (6) 994-1000. Journal code: 0370507. ISSN: 0002-9637. Pub. country: United States. Language: English.

AB This study describes the use of the reverse transcriptase-polymerase chain reaction (RT-**PCR**) to generate dengue 2 amplicons from paraffin-embedded autopsy tissues collected in Cuba 17 years ago. The presumptive diagnoses had been made only by clinical evolution without serologic confirmation. This study confirms once again that dengue 2 virus was directly associated with the fatal cases in children and illustrates the potential of the RT-**PCR** for retrospective diagnosis of dengue cases 17 years after death. A close similarity in the genomic sequences of the dengue 2 RNA detected in tissue samples from fatal cases and those dengue 2 Cuban strains that had been previously investigated confirms the appropriate genomic classification of the etiologic agent associated with the 1981 dengue hemorrhagic fever Cuban epidemic.

L15 ANSWER 21 OF 65 MEDLINE on STN

2000052185. PubMed ID: 10586902. Common occurrence of concurrent infections by multiple **dengue virus** serotypes. Lorono-Pino M A; Cropp C B; Farfan J A; Vorndam A V; Rodriguez-Angulo E M; Rosado-Paredes E P; Flores-Flores L F; Beaty B J; Gubler D J. (Centro de Investigaciones Regionales Dr. Hideyo Noguchi, Universidad Autonoma de Yucatan, Merida, Mexico.) American journal of tropical medicine and hygiene, (1999 Nov) 61 (5) 725-30. Journal code: 0370507. ISSN: 0002-9637. Pub. country: United States. Language: English.

AB The co-circulation of all 4 **dengue virus** serotypes in the same community, common since the 1950s in Southeast Asia, has now become a frequent occurrence in many Caribbean Islands, Mexico, and Central and South America in the past 20 years. As a consequence, the frequency of concurrent infections would be expected to increase in these areas. To assess this, using state of the art technology, we screened viremic serum samples and mosquitoes inoculated with serum samples collected during epidemics involving multiple **dengue virus** serotypes in Indonesia, Mexico, and Puerto Rico for virus isolation. Of 292 samples tested, 16 (5.5%) were found to contain 2 or more dengue viruses by an indirect immunofluorescence test and/or the reverse transcriptase-polymerase chain reaction.

L15 ANSWER 22 OF 65 MEDLINE on STN

2000052184. PubMed ID: 10586901. Detection of **dengue virus** RNA by reverse transcription-polymerase chain reaction in the liver and lymphoid organs but not in the brain in fatal human infection. Rosen L; Drouet M T; Deubel V. (Unite d'Ecologie des Systemes Vectoriels, Institut Pasteur, Paris, France.) American journal of tropical medicine and hygiene, (1999 Nov) 61 (5) 720-4. Journal code: 0370507. ISSN: 0002-9637. Pub. country: United States. Language: English.

AB Autopsy tissues from 18 children believed to have died of dengue hemorrhagic fever were tested for the presence of **dengue virus** RNA by reverse transcription-polymerase chain reaction (RT-**PCR**). Such RNA was found in 14 of 18 liver specimens, 13 of 18 spleen specimens and 7 of 16 mesenteric lymph node specimens. No **dengue virus** RNA was detected in 44 samples of brain tissue from 15 individuals, 1 or more of whose other tissues yielded such RNA. All tissues had been tested previously for **dengue virus** by mosquito inoculation. In those tests, virus was recovered from 5 of 18 liver and 2 of 18 spleen specimens. Thus, the RT-**PCR** is more sensitive than the most sensitive virus isolation technique for detecting **dengue virus** or its components in human tissue. Failure to isolate virus from most of spleen and all mesenteric lymph node specimens may indicate that those tissues contained

L15 ANSWER 23 OF 65 MEDLINE on STN

1999335533. PubMed ID: 10405398. Detection of **dengue virus** RNA in patients after primary or secondary dengue infection by using the TaqMan automated amplification system. Laue T; Emmerich P; Schmitz H. (Department of Virology, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany.) Journal of clinical microbiology, (1999 Aug) 37 (8) 2543-7. Journal code: 7505564. ISSN: 0095-1137. Pub. country: United States. Language: English.

AB In consecutive serum samples from 25 tourists with acute dengue fever, virus-specific RNA was detected by using fully automated TaqMan reverse transcriptase **PCR**. For this amplification technique new **primers** and special fluorochrome-labeled probes had to be synthesized. During amplification the increasing amount of viral DNA could simultaneously be measured in the tightly sealed tubes. **Dengue virus** RNA was found in almost all patients (17 of 18), if the samples had been taken soon after the onset of symptoms and before anti-**dengue virus** antibody had been produced. RNA was detectable in only one of five persons who had anti-**dengue virus** immunoglobulin M (IgM) antibodies but not yet IgG antibodies. In 30 late samples with both IgG and IgM antibodies viral RNA was no longer demonstrable. In two early samples from two frequent travelers obtained 1 and 2 days after the onset of symptoms significant IgG antibody titers were present but there were no anti-**dengue virus** IgM antibodies. In these samples a viral load of $>5 \times 10^6$ **dengue virus** RNA copies (dengue types 1 and 2) was detectable. These findings of a high viral load in the presence of anti-**dengue virus** IgG antibody are suggestive of a secondary **dengue virus** infection. In the 20 tourists (17 plus 1 plus 2) in whom viral RNA was found, the **dengue virus** serotype could be related to the area where the infection had taken place. Most of our patients came from southeast Asia and most frequently had **dengue virus** type 1 infections (8 of 20).

L15 ANSWER 24 OF 65 MEDLINE on STN

1999330112. PubMed ID: 10403318. Type-3 dengue viruses responsible for the dengue epidemic in Malaysia during 1993-1994. Kobayashi N; Thayan R; Sugimoto C; Oda K; Saat Z; Vijayamalar B; Sinniah M; Igarashi A. (Division of Microbiology, Yokohama City Institute of Health, Yokohama, Japan.) American journal of tropical medicine and hygiene, (1999 Jun) 60 (6) 904-9. Journal code: 0370507. ISSN: 0002-9637. Pub. country: United States. Language: English.

AB To characterize the dengue epidemic that recently occurred in Malaysia, we sequenced cDNAs from nine 1993-1994 **dengue virus** type-3 (DEN-3) isolates in Malaysia (DEN-3 was the most common type in Malaysia during this period). Nucleic acid sequences (720 nucleotides in length) from the nine isolates, encompassing the precursor of membrane protein (preM) and membrane (M) protein genes and part of the envelope (E) protein gene were aligned with various reference DEN-3 sequences to generate a neighbor-joining phylogenetic tree. According to the constructed tree, the nine Malaysian isolates were grouped into subtype II, which comprises Thai isolates from 1962 to 1987. Five earlier DEN-3 virus Malaysian isolates from 1974 to 1981 belonged to subtype I. The present data indicate that the recent dengue epidemic in Malaysia was due to the introduction of DEN-3 viruses previously endemic to Thailand.

L15 ANSWER 25 OF 65 MEDLINE on STN

1999162696. PubMed ID: 10063209. A Japanese case of dengue fever with lymphocytic vasculitis: diagnosis by **polymerase chain reaction**. Ishikawa H; Okada S; Katayama I; Mazaki H; Nagatake T; Hasebe F; Igarashi A. (Department of Dermatology, Nagasaki University School of Medicine, Japan.) Journal of dermatology, (1999 Jan) 26 (1) 29-32. Journal code: 7600545. ISSN: 0385-2407. Pub. country: Japan. Language: English.

AB A 37-year-old Japanese male was admitted to Nagasaki University Hospital with abrupt onset of biphasic fever, general malaise and myalgia 9 days after coming back to Japan from Manila. He developed a rubella like erythematous rash 3 days after admission and purpuric eruption one week after admission. A biopsied specimen from the purpura revealed lymphocytic vasculitis with T cell dominance and without immunoglobulin or complement deposition around the blood vessels. RT-**PCR** analysis on peripheral blood mononuclear cells using **dengue virus** specific **primers** confirmed the diagnosis of type 3 dengue fever. **PCR** analysis using virus specific **primers** is a rapid and valuable method for making a correct diagnosis of dengue fever.

L15 ANSWER 26 OF 65 MEDLINE on STN

1999124791. PubMed ID: 9887321. Rapid subtyping of dengue viruses by restriction site-specific (RSS)-**PCR**. Harris E; Sandoval E; Xet-Mull A M; Johnson M; Riley L W. (Division of Public Health Biology and Epidemiology, University of California, Berkeley, California, 94720, USA.. eharris@socrates.berkeley.edu) . Virology, (1999 Jan 5) 253 (1) 86-95. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB Dengue is a major public health problem worldwide. It is caused by four

subtypes. Strain typing is important for understanding the epidemiology and viral factors associated with disease transmission. However, most of the existing subtyping methods are expensive and technically unwieldy for timely, practical applications in developing countries. Here we describe a simple, rapid, **PCR**-based subtyping method, restriction site-specific (RSS)-**PCR**, which we used to analyze **dengue virus** serotypes 2 and 3. For each serotype, four **primers** targeted to sequences spanning polymorphic endonuclease restriction sites in the envelope gene were used to reverse transcribe and amplify viral RNA. These RT-**PCR** products generated distinct electrophoretic band patterns for different strains. Analysis of 73 dengue-2 strains and 54 dengue-3 strains representing a broad geographic distribution over several decades revealed that the RSS-**PCR** fingerprints reproducibly fell into 7 and 3 groups, respectively. These groups correlated well with previous phylogenetic classifications. This one-step assay should be widely accessible and allow more detailed epidemiologic investigations in dengue-endemic countries. This novel **PCR** approach to subtyping organisms based on restriction site polymorphisms should be applicable to other pathogens. Copyright 1999 Academic Press.

L15 ANSWER 27 OF 65 MEDLINE on STN

1999101219. PubMed ID: 9886128. Nucleotide sequence and deduced amino acid sequence of the nonstructural proteins of dengue type 3 virus, Bangkok genotype. Attatippaholkun W H; Attatippaholkun M K; Nisalak A; Vaughn D W; Innis B L. (Department of Clinical Chemistry, Faculty of Medical Technology, Mahidol University, Bangkok, Thailand.) Southeast Asian journal of tropical medicine and public health, (1998 Jun) 29 (2) 361-6. Journal code: 0266303. ISSN: 0125-1562. Pub. country: Thailand. Language: English.

AB The nucleotide sequence of the nonstructural protein gene (1,610 bases) of dengue 3 virus (Bangkok genotype; CH53489 isolated in 1973) has been determined in both forward and reverse directions. The **PCR** based cycle sequencing technic by the enzymatic method of Sanger et al using a sequencing **primer** 5'-end labeled with gamma-32P-ATP was the method of our choice for sequence analysis. Two cDNA templates were prepared by RT-**PCR** technique starting from the nucleotides 6,306-6,969 and 6,925-7,915 of the dengue 3 genome with the lengths of 663 and 990 base pairs respectively. In our cycle sequencing experiments, it has been observed that the substitution of 7-deaza-dG for dG in DNA eliminated most of the secondary structures that produce gel artifacts. The final sequence results of these two cDNA templates were established from their sequence data determined on both strands in opposite directions. Alignment between the newly established nucleotide sequences as well as their deduced amino acid sequences of the Bangkok dengue 3 (CH53489) virus and the published sequence data of the dengue 3 prototype (H87) was manipulated by the PC-DOS-GIBIO DNASIS TM 06-00 software. The homology of the nucleotide sequences between the two dengue 3 viruses was 96.65%. The deduced amino acid sequence from nucleotides 6,306-7,915 of the two viruses showed conserved amino acids of the nonstructural protein NS4a and 6 amino acid changes in NS4b and NS5.

L15 ANSWER 28 OF 65 MEDLINE on STN

1999021856. PubMed ID: 9805040. [The rapid identification of **dengue virus** serotypes by the **polymerase chain reaction**]. Identificacion rapida de los serotipos del virus del dengue mediante la reaccion en cadena de la polimerasa. Rosario Dominguez D; Suarez Moran C M; Rodriguez Roche R; Soler Nodarse M; Guzman Tirado M G. (Instituto de Medicina Tropical Pedro Kouri.) Revista cubana de medicina tropical, (1996) 48 (3) 155-60. Journal code: 0074364. ISSN: 0375-0760. Pub. country: Cuba. Language: Spanish.

AB 4 **primer** sets, were used to allow the amplification of a nucleotide sequence with unique size for each of the **dengue virus** serotypes by **polymerase chain reaction** (PRC). The method consisted in the extraction of ribonucleic acid from supernatant of infected cell cultures, reverse transcription-**polymerase chain reaction** (RT-**PCR**). This was completed in approximately 7 hours in a simple tube. The size of the amplified sequence was evidenced by electrophoresis in Agarose gel stained with ethidium. The method showed a sensitivity of at least 2.5 plate forming units (pfu) per tube of reaction. It es useful for the detection and simultaneous identification of the 4 serotypes, starting from supernatants of infected strains cultures from different countries of the Caribbean, Central America, and South America.

L15 ANSWER 29 OF 65 MEDLINE on STN

1998438284. PubMed ID: 9766894. Microplate-reverse hybridization method to determine **dengue virus** serotype. Sudiro T M; Ishiko H; Rothman A L; Kershaw D E; Green S; Vaughn D W; Nisalak A; Kalayanarooj S; Ennis F A. (Center for Infectious Disease and Vaccine Research, University of Massachusetts Medical School, Worcester 01655, USA.) Journal of virological methods, (1998 Aug) 73 (2) 229-35. Journal code: 8005839. ISSN: 0166-0934. Pub. country: Netherlands. Language: English.

AB A reverse transcriptase-**polymerase chain reaction** (RT-**PCR**) and

dengue viruses in patients plasma specimens. A silica method was used to isolate RNA; and 3'-noncoding region universal **primers** were used to amplify **dengue virus** RNA. Using RT-PCR and ethidium bromide staining we could detect **dengue virus** in serum spiked with serially diluted **dengue virus** with a level of sensitivity similar to that of a quantitative fluorescent focus assay of dengue viruses in cell culture, i.e. 1.4 fluorescent focus units per reaction. Applying this assay to 14 dengue-positive plasma samples and 13 dengue-negative samples, dengue viremia was detectable by RT-PCR with a sensitivity comparable to mosquito inoculation. To determine the serotypes, digoxigenin-labeled PCR products from plasma samples and six laboratory adapted dengue viruses were hybridized in stringent conditions to serotype-specific DNA probes immobilized on microplates, and the hybridized product was detected with a colorimetric assay. Serotypes of dengue viruses, in cell culture and in patient plasma specimens, were identified using this method.

L15 ANSWER 30 OF 65 MEDLINE on STN

1998420054. PubMed ID: 9749625. Identification of Brazilian flaviviruses by a simplified reverse transcription-**polymerase chain reaction** method using Flavivirus universal **primers**. Figueiredo L T; Batista W C; Kashima S; Nassar E S. (Unidade Multidisciplinar de Pesquisa em Virologia, Faculdade de Medicina de Ribeirao Preto, Universidade de Sao Paulo, SP, Brazil.) American journal of tropical medicine and hygiene, (1998 Sep) 59 (3) 357-62. Journal code: 0370507. ISSN: 0002-9637. Pub. country: United States. Language: English.

AB We report a simplified reverse transcription-**polymerase chain reaction** (RT-PCR) method for identification of Brazilian flaviviruses based on the patterns of electrophoretic separation of the amplicons. The RT-PCR was done on the culture fluids of Aedes albopictus C6/36 cells infected with Brazilian flaviviruses, without previous extraction of viral RNA, using Flavivirus universal **primers** that anneal to highly conserved sequences within the nonstructural protein 5 and 3'- non translated region of the virus genome. Genomes of 13 Brazilian Flavivirus isolates were amplified. It was not possible to amplify the genome of Bussuquara virus. Analysis of the RT-PCR products gave reproducible results and three distinct amplicon patterns were observed. Cacipacore (800-850 basepairs [bp]) and yellow fever viruses (600 bp) yielded a single amplicon; **dengue virus** types 1 and 2 (650 and 550 bp), **dengue virus** type 4 (550 and 450 bp), Iguape (650-600 bp and 750-700 bp), St. Louis encephalitis (700 and 650-600 bp), and Rocio viruses (600 and 500-550 bp) yielded two amplicons; and Ilheus virus yielded five amplicons, two larger than 1,000 bp, one 650-700 bp, one 550-600 bp, and one 450-500 bp. The analysis of amplicon DNA sequences of six viruses showed homology with the 3'- nontranslated region of Flavivirus genome. The use of the Flavivirus universal **primers** in this simple RT-PCR technique is suitable as a screening test for the genus Flavivirus, with the exception of Bussuquara virus, in Brazilian isolates in tissue culture fluid.

L15 ANSWER 31 OF 65 MEDLINE on STN

1998404732. PubMed ID: 9734221. [Polymerase chain reaction for rapid detection and serotyping of **dengue virus** in clinical samples]. Reaccion en cadena de la polimerasa para la deteccion rapida y determinacion del serotipo de virus del dengue en muestras clinicas. Rosario D; Alvarez M; Diaz J; Contreras R; Rodriguez R; Vazquez S; Guzman M G. (Instituto de Medicina Tropical Pedro Kouri, La Habana, Cuba.) Revista panamericana de salud publica = Pan American journal of public health, (1998 Jul) 4 (1) 1-5. Journal code: 9705400. ISSN: 1020-4989. Pub. country: United States. Language: Spanish.

AB This study describes the benefits of using reverse transcriptase **polymerase chain reaction** (RT-PCR) for the rapid detection and typing of **dengue virus** in clinical samples. Twenty-seven serum specimens from patients with dengue fever and dengue hemorrhagic fever in Colombia, Nicaragua, and Panama were directly subjected to RT-PCR for the detection of **dengue virus**. The resulting double-stranded DNA product was typed by a second round of PCR amplification (nested PCR) with type-specific **primers**, viral culture/indirect immunofluorescence (IIF), and enzyme-linked electroimmunoassay for IgM anti-dengue antibodies. The amplified virus genome was detected and typed within 8 hours. Nested RT-PCR, using viral culture and IIF as the gold standard, showed 100% sensitivity; 78% specificity; 69% positive predictive value, and 100% negative predictive value. It is noteworthy that two of the specimens whose results were positive with nested RT-PCR and negative with viral culture showed specific IgM antibodies. The results of the RT-PCR were in close agreement with those obtained through viral culture. This suggests PCR can greatly facilitate the rapid and early diagnosis of dengue infection.

L15 ANSWER 32 OF 65 MEDLINE on STN

1998371106. PubMed ID: 9705406. Typing of dengue viruses in clinical specimens and mosquitoes by single-tube multiplex reverse transcriptase PCR. Harris E; Roberts T G; Smith L; Selle J; Kramer L D; Valle S; Sandoval E; Balmaseda A. (Program in Molecular Pathogenesis, University of

eharris@uclink4.berkeley.edu) . Journal of clinical microbiology, (1998 Sep) 36 (9) 2634-9. Journal code: 7505564. ISSN: 0095-1137. Pub. country: United States. Language: English.

AB In recent years, dengue viruses (serotypes 1 to 4) have spread throughout tropical regions worldwide. In many places, multiple **dengue virus** serotypes are circulating concurrently, which may increase the risk for the more severe form of the disease, dengue hemorrhagic fever. For the control and prevention of dengue fever, it is important to rapidly detect and type the virus in clinical samples and mosquitoes. Assays based on reverse transcriptase (RT) **PCR** (RT-**PCR**) amplification of dengue viral RNA can offer a rapid, sensitive, and specific approach to the typing of dengue viruses. We have reduced a two-step nested RT-**PCR** protocol to a single-tube reaction with sensitivity equivalent to that of the two-step protocol (1 to 50 PFU) in order to maximize simplicity and minimize the risk of sample cross-contamination. This assay was also optimized for use with a thermostable RT-polymerase. We designed a plasmid-based internal control that produces a uniquely sized product and can be used to control for both reverse transcription or amplification steps without the risk of generating false-positive results. This single-tube RT-**PCR** procedure was used to type dengue viruses during the 1995 and 1997-1998 outbreaks in Nicaragua. In addition, an extraction procedure that permits the sensitive detection of viral RNA in pools of up to 50 mosquitoes without **PCR** inhibition or RNA degradation was developed. This assay should serve as a practical tool for use in countries where dengue fever is endemic, in conjunction with classical methods for surveillance and epidemiology of dengue viruses.

L15 ANSWER 33 OF 65 MEDLINE on STN
1998258391. PubMed ID: 9598444. Monitoring of dengue viruses in field-caught *Aedes aegypti* and *Aedes albopictus* mosquitoes by a type-specific **polymerase chain reaction** and cycle sequencing. Chow V T; Chan Y C; Yong R; Lee K M; Lim L K; Chung Y K; Lam-Phua S G; Tan B T. (Department of Microbiology, Faculty of Medicine, National University of Singapore, Kent Ridge.) American journal of tropical medicine and hygiene, (1998 May) 58 (5) 578-86. Journal code: 0370507. ISSN: 0002-9637. Pub. country: United States. Language: English.

AB Virologic surveillance for dengue through the detection of the prevalent serotype(s) circulating in the human population during inter- and intra-epidemic periods constitutes a reliable sentinel system for dengue outbreaks. We have applied a rapid and sensitive, semi-nested, reverse transcription-**polymerase chain reaction** (RT-**PCR**) assay using nonstructural protein 3 gene **primers** for the type-specific-detection of dengue viruses in artificially infected and in field-caught adult *Aedes* mosquitoes. In laboratory experiments, the assay was sensitive enough to detect one virus-infected mosquito head in pools of up to 59 uninfected heads. In a prospective field study conducted from April 1995 to July 1996, female adult *Ae. aegypti* and *Ae. albopictus* mosquitoes were caught from selected dengue-sensitive areas in Singapore and assayed by RT-**PCR**. Approximately 20% of 309 mosquito pools were positive for dengue viruses. Of the 23 RT-**PCR**-positive *Ae. aegypti* pools (containing 1-17 mosquitoes each), 18 pools (78.3%) were positive for dengue 1 virus. There were 40 RT-**PCR**-positive *Ae. albopictus* pools (containing 1-33 mosquitoes each) of which 31 (77.5%) were positive for dengue 1 virus. The predominant virus type responsible for the current dengue epidemic since 1995 was also dengue 1. The geographic locations of the virus-infected mosquitoes correlated with the residences or workplaces of patients within dengue outbreak areas. A total of 43.5% of the positive *Ae. aegypti* pools and 25.0% of the positive *Ae. albopictus* pools contained only a single mosquito. Both *Aedes* species showed similar overall minimum infection rates of 57.6 and 50 per 1,000 mosquitoes. Infected *Ae. aegypti* were detected as early as six weeks before the start of the dengue outbreaks in 1995 and 1996. However, infected *Ae. albopictus* appeared later, when the number of cases was increasing. Virologic surveillance by RT-**PCR** for detecting **dengue virus**-infected *Aedes* mosquitoes in the field may serve as an early warning monitoring system for dengue outbreaks.

L15 ANSWER 34 OF 65 MEDLINE on STN
1998227918. PubMed ID: 9568963. Definition of the region on NS3 which contains multiple epitopes recognized by **dengue virus** serotype-cross-reactive and flavivirus-cross-reactive, HLA-DPw2-restricted CD4+ T cell clones. Okamoto Y; Kurane I; Leporati A M; Ennis F A. (Center for Infectious Disease and Vaccine Research, Department of Medicine, University of Massachusetts Medical Center, Worcester 01655, USA.) Journal of general virology, (1998 Apr) 79 (Pt 4) 697-704. Journal code: 0077340. ISSN: 0022-1317. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The epitopes recognized by six CD4+ CD8- cytotoxic T lymphocyte (CTL) clones established from a dengue-3 virus-immune donor were defined. (i) Three CTL clones, JK10, JK34 and JK39, were cross-reactive for **dengue virus** types 1-4. (ii) One clone, JK28, was cross-reactive for **dengue virus** types 1-4 and West Nile virus. (iii) Two clones, JK26 and JK49, were cross-reactive for **dengue virus** types 1-4, West Nile virus and

epitope on NS3 in an HLA-DPw2-restricted fashion. The smallest synthetic peptide recognized by the five CTL clones was a 10 aa peptide which comprises aa 255-264 on **dengue virus** NS3. JK49 recognized the overlapping epitope which comprises aa 257-266 in an HLA-DPw2-restricted fashion. Analysis of T cell receptor (TCR) usage by these T cell clones revealed that (i) JK10 and JK34 use V alpha11, and JK34 and JK28 use V beta23, and (ii) the amino acid sequences of the V(D)J junctional region of the TCR were different among these five CTL clones. There were, however, single amino acid conservations among TCRs of some of these T cell clones. These results indicate that the region on NS3 which comprises aa 255-266 contains multiple epitopes recognized by dengue serotype-cross-reactive and flavivirus-cross-reactive CD4+ CTL in an HLA-DPw2-restricted fashion and that a single epitope can be recognized by T cells which have heterogeneous virus specificities.

L15 ANSWER 35 OF 65 MEDLINE on STN

1998183587. PubMed ID: 9522986. Molecular diagnosis and epidemiology of **dengue virus** infection. Chow V T. (Department of Microbiology, National University of Singapore, Singapore.) Annals of the Academy of Medicine, Singapore, (1997 Nov) 26 (6) 820-6. Journal code: 7503289. ISSN: 0304-4602. Pub. country: Singapore. Language: English.

AB Early diagnosis of dengue fever contributes towards appropriate management of the disease and its potentially severe complications. Better and more rapid molecular procedures are increasingly available for detecting dengue viral RNA, antibodies and antigens. Using consensus **primers** based on the conserved non-structural-3 gene, the reverse transcription-**polymerase chain reaction** (RT-PCR) technique can amplify all four **dengue virus** types as well as certain flaviviruses. Consensus **primers** used together with four type-specific downstream **primers** in single-step or semi-nested RT-PCR formats can discriminate the specific **dengue virus** type by virtue of the diagnostic size of the RT-PCR target fragment on agarose gel electrophoresis. Alternatively, RT-PCR products may be labelled with digoxigenin and allowed to hybridise with individual biotinylated type-specific **PCR primers** which act as capture probes immobilised on solid phase via streptavidin-coated tubes. With automated instrumentation for enzyme-linked immunosorbent assay (ELISA), the hybridised RT-PCR products can be quantitated spectrophotometrically via anti-digoxigenin antibodies conjugated with an enzyme which reacts with colourimetric substrate. While RT-PCR is highly sensitive, specific and successfully identifies the **dengue virus** type in clinical serum samples and adult Aedes mosquitoes, it generally yields positive results in viraemic sera collected within 2 to 5 days of pyrexia. Sera obtained after the period of viraemia are more likely to be positive by serological tests such as IgM capture ELISA or the commercial Dengue Blot kit. The RT-PCR **primers** can also be utilised for direct cycle dideoxy DNA sequencing to monitor the molecular epidemiology and evolution of geographically and temporally separated virus strains. To exemplify this, nucleotide and amino acid sequence data as well as phylogenetic trees of several strains of dengue 1 and 2 viruses from patients and field-caught Aedes mosquitoes are presented.

L15 ANSWER 36 OF 65 MEDLINE on STN

1998056385. PubMed ID: 9394519. Detection and identification of **dengue virus** isolates from Brazil by a simplified reverse transcription-**polymerase chain reaction** (RT-PCR) method. Figueiredo L T; Batista W C; Igarashi A. (Unidade Multidisciplinar de Pesquisa em Virologia, Faculdade de Medicina de Ribeiro Preto, Universidade de Sao Paulo, Brasil.) Revista do Instituto de Medicina Tropical de Sao Paulo, (1997 Mar-Apr) 39 (2) 79-83. Journal code: 7507484. ISSN: 0036-4665. Pub. country: Brazil. Language: English.

AB We show here a simplified RT-PCR for identification of **dengue virus** types 1 and 2. Five **dengue virus** strains, isolated from Brazilian patients, and yellow fever vaccine 17DD as a negative control, were used in this study. C6/36 cells were infected and supernatants were collected after 7 days. The RT-PCR, done in a single reaction vessel, was carried out following a 1/10 dilution of virus in distilled water or in a detergent mixture containing Nonidet P40. The 50 microliters assay reaction mixture included 50 pmol of specific **primers** amplifying a 482 base pair sequence for dengue type 1 and 210 base pair sequence for dengue type 2. In other assays, we used **dengue virus** consensus **primers** having maximum sequence similarity to the four serotypes, amplifying a 511 base pair sequence. The reaction mixture also contained 0.1 mM of the four deoxynucleoside triphosphates, 7.5 U of reverse transcriptase, 1U of thermostable Taq DNA polymerase. The mixture was incubated for 5 minutes at 37 degrees C for reverse transcription followed by 30 cycles of two-step **PCR** amplification (92 degrees C for 60 seconds, 53 degrees C for 60 seconds) with slow temperature increment. The **PCR** products were subjected to 1.7% agarose gel electrophoresis and visualized by UV light after staining with ethidium bromide solution. Low virus titer around 10(3, 6) TCID50/ml was detected by RT-PCR for dengue type 1. Specific DNA amplification was observed with all the Brazilian dengue strains by using **dengue virus** consensus **primers**. As compared to other RT-PCRs,

of contamination.

L15 ANSWER 37 OF 65 MEDLINE on STN

97473765. PubMed ID: 9332607. A simple reverse transcription-**polymerase chain reaction** for dengue type 2 virus identification. Figueiredo L T; Batista W C; Igarashi A. (Unidade Multidisciplinar de Pesquisa em Virologia, Faculdade de Medicina de Ribeirao Preto, Universidade de Sao Paulo, Brasil.) Memorias do Instituto Oswaldo Cruz, (1997 May-Jun) 92 (3) 395-8. Journal code: 7502619. ISSN: 0074-0276. Pub. country: Brazil. Language: English.

AB We show here a simplified reverse transcription-**polymerase chain reaction** (RT-PCR) for identification of dengue type 2 virus. Three dengue type 2 virus strains, isolated from Brazilian patients, and yellow fever vaccine 17DD, as a negative control, were used in this study. C6/36 cells were infected with the virus, and tissue culture fluids were collected after 7 days of infection period. The RT-PCR, a combination of RT and PCR done after a single addition of reagents in a single reaction vessel was carried out following a digestion of virus with 1% Nonidet P-40. The 50 microliters assay reaction mixture included 50 pmol of a dengue type 2 specific **primer** pair amplifying a 210 base pair sequence of the envelope protein gene, 0.1 mM of the four deoxynucleoside triphosphates, 7.5 U of reverse transcriptase, and IU of thermostable Taq DNA polymerase. The reagent mixture was incubated for 15 min at 37 degrees C for RT followed by a variable amount of cycles of two-step PCR amplification (92 degrees C for 60 sec, 53 degrees C for 60 sec) with slow temperature increment. The PCR products were subjected to 1.7% agarose gel electrophoresis and visualized with UV light after gel incubation in ethidium bromide solution. DNA bands were observed after 25 and 30 cycles of PCR. Virus amount as low as 10(2.8) TCID 50/ml was detected by RT-PCR. Specific DNA amplification was observed with the three dengue type 2 strains. This assay has advantages compared to other RT-PCRs: it avoids laborious extraction of virus RNA; the combination of RT and PCR reduces assay time, facilitates the performance and reduces risk of contamination; the two-step PCR cycle produces a clear DNA amplification, saves assay time and simplifies the technique.

L15 ANSWER 38 OF 65 MEDLINE on STN

97425933. PubMed ID: 9279982. Rapid detection and identification of dengue viruses by **polymerase chain reaction** (PCR). Yenchitsomanus P T; Sricharoen P; Jaruthasana I; Pattanakitsakul S N; Nitayaphan S; Mongkolsapaya J; Malasit P. (Medical Molecular Biology Unit, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand.) Southeast Asian journal of tropical medicine and public health, (1996 Jun) 27 (2) 228-36. Journal code: 0266303. ISSN: 0125-1562. Pub. country: Thailand. Language: English.

AB A **polymerase chain reaction** (PCR) method using sets of newly designed **primers** for rapid detection and simultaneous identification of **dengue virus** serotypes was developed and tested. The test is based on two sets of **primers** specific within the envelope (E) and non-structural (NS1) regions of the **dengue-virus** genome. Two sets of universal **primers** that bind to two target sequences which are shared by all the four serotypes of the virus within the E and NS1 regions are used. The resulting products are further amplified by another pair of inner or nested universal **primers**, which also bind to another set of shared sequences within the E and NS1 regions, respectively. The nested PCR of both the E and NS1 regions can detect **dengue virus** of all the four serotypes at a sensitivity of 1 plaque forming unit (pfu) or less. For the identification of serotypes, a mixture of four pairs of serotype-specific **primers**, specific to the E region, was used. The **primers** have been designed to bind to serotype specific sequences within the regions flanked by the outer universal **primers**, and giving the amplified products of different sizes, each corresponds to one particular serotype (405 bp for Den1, 346 bp for Den2, 196 bp for Den3, and 143 bp for Den4). A protocol has been developed and successfully applied to detect **dengue virus** in cell-culture supernatants and patients sera. The technique is simple and rapid, capable of not only detecting the **dengue virus** but also identifying the serotypes of the virus in clinical specimens.

L15 ANSWER 39 OF 65 MEDLINE on STN

97352170. PubMed ID: 9208450. Sequences of terminal non-coding regions from four dengue-2 viruses isolated from patients exhibiting different disease severities. Mangada M N; Igarashi A. (Department of Virology, Institute of Tropical Medicine, Nagasaki University, Japan.) Virus genes, (1997) 14 (1) 5-12. Journal code: 8803967. ISSN: 0920-8569. Pub. country: United States. Language: English.

AB We have determined the 5' and 3' non-coding regions (NCR) of four dengue-2 viruses isolated from dengue patients with different clinical severities in Nakhon Phanom, Northeastern Thailand in 1993. The results were compared to prototype dengue-2 strains and were found to have highest homology with the New Guinea C strain. The sequence of the 5' NCR was completely conserved among all 4 isolates and were identical to the

the four isolates with the prototype strain ranged from 97.3 to 97.8%. Isolate ThNH-p11/93 from a mild dengue fever case showed the highest divergence from the prototype strain and the rest of the isolates from severe hemorrhagic cases, (1.11%). This includes a change in triad 297-299 nucleotides from the 3' terminus. Computer predicted secondary structures showed that isolate ThNHp-11/93 had significant structural differences from the other three isolates at this region.

L15 ANSWER 40 OF 65 MEDLINE on STN

97301651. PubMed ID: 9158052. Rapid diagnosis of dengue viremia by reverse transcriptase-**polymerase chain reaction** using 3'-noncoding region universal **primers**. Sudiro T M; Ishiko H; Green S; Vaughn D W; Nisalak A; Kalayanaroj S; Rothman A L; Raengsakulrach B; Janus J; Kurane I; Ennis F A. (Division of Infectious Diseases and Immunology, University of Massachusetts Medical School, Worcester 01655, USA.) American journal of tropical medicine and hygiene, (1997 Apr) 56 (4) 424-9. Journal code: 0370507. ISSN: 0002-9637. Pub. country: United States. Language: English.

AB A reverse transcriptase-**polymerase chain reaction** (RT-PCR) method was developed as a rapid diagnostic test of dengue viremia. To detect dengue viruses in serum or plasma specimens, a pair of universal **primers** was designed for use in the RT-PCR. Using these **primers**, the 3'-noncoding region of **dengue virus** types 1, 2, 3, and 4 could be amplified, but not those of other flaviviruses, such as West Nile virus, Japanese encephalitis virus, and yellow fever virus, or the alphavirus Sindbis virus. The sensitivity of the RT-PCR assay was similar to that of a quantitative fluorescent focus assay of dengue viruses in cell culture. Combining a silica method for RNA isolation and RT-PCR **dengue virus** could be detected in a 6-hr assay. In a preliminary study using this method, we detected **dengue virus** in 38 of 39 plasma specimens from which **dengue virus** had been isolated by mosquito inoculation. We then applied this method for detecting dengue viremia to 117 plasma samples from 62 children with acute febrile illnesses in a dengue-endemic area. We detected dengue viremia in 19 of 20 samples obtained on the day of presentation, which had been confirmed as acute dengue infection by mosquito inoculation and antibody responses. The overall sensitivity of this method was 91.4% (32 of 35; 95% confidence interval [CI] = 82.2-100%). The results from testing plasma samples from febrile nondengue patients showed a specificity of 95.4% (42 of 44; 95% CI = 89.3-100%).

L15 ANSWER 41 OF 65 MEDLINE on STN

97276479. PubMed ID: 9130232. Detection of flaviviruses by reverse transcriptase-**polymerase chain reaction** with the universal **primer** set. Meiyu F; Huosheng C; Cuihua C; Xiaodong T; Lianhua J; Yifei P; Weijun C; Huiyu G. (Medical Research Institute, Yan-ling, Guangzhou, China.) Microbiology and immunology, (1997) 41 (3) 209-13. Journal code: 7703966. ISSN: 0385-5600. Pub. country: Japan. Language: English.

AB Using a universal **primer** set designed to match the sequence of the NS1 gene of flaviviruses, the virus RNA of dengue (DEN), Japanese encephalitis (JEV), powassan and langat of Flaviviridae were successfully amplified by **polymerase chain reaction** (PCR) via cDNA; and with different internal **primers**, the serotypes of the dengue viruses were identified. Of the 78 clinically diagnosed dengue fever patients, 18 patients were positive for DEN 1, 48 patients for DEN 2 and 8 patients concurrently infected with DEN 4. Of the 52 patients admitted with Japanese encephalitis (JE), 45 were determined to be JEV infections. By nested PCR, we completed the identification of flaviviruses within 2 days. The results show that seven **primers** have a potential value for rapid clinical diagnosis of flavivirus infections.

L15 ANSWER 42 OF 65 MEDLINE on STN

97209777. PubMed ID: 9056952. Nucleotide sequences from the capsid and pre-protein regions of dengue viruses from VietNam. Nguyen N H; Tran V B; Morris G E. (MRIC Biochemistry Group, N.E. Wales Institute, UK.) Biochemical Society transactions, (1997 Feb) 25 (1) 54S. Journal code: 7506897. ISSN: 0300-5127. Pub. country: ENGLAND: United Kingdom. Language: English.

L15 ANSWER 43 OF 65 MEDLINE on STN

97206455. PubMed ID: 9139373. The use of **polymerase chain reaction** (PCR) as a diagnostic tool for **dengue virus**. Thayan R; Vijayamalar B; Zainah S; Chew T K; Morita K; Sinniah M; Igarashi A. (Division of Virology, Institute for Medical Research, Jalan Pahang, Kuala Lumpur, Malaysia.) Southeast Asian journal of tropical medicine and public health, (1995 Dec) 26 (4) 669-72. Journal code: 0266303. ISSN: 0125-1562. Pub. country: Thailand. Language: English.

AB This study describes the use of **polymerase chain reaction** as a diagnostic tool for detecting and typing of **dengue virus**. PCR was compared against virus isolation. First RT-PCR was done using dengue consensus **primers** after which positive samples were subjected to RT-PCR using type-specific **primers**. This study shows that the local strains of the **dengue virus** could be detected using the chosen

virus isolation in identifying the dengue positive samples.

L15 ANSWER 44 OF 65 MEDLINE on STN

97206454. PubMed ID: 9139372. Genotype determination of three dengue type 2 virus strains from Myanmar by sequencing E/NSI gene junction. Kyaw-Zin-Thant; Khin-Mar-Aye; Soe-Thein; Than-Swe; Hasebe F; Morita K; Igarashi A. (Department of Virology, Nagasaki University, Japan. igarashi@net.nagasaki-u.ac.jp.) Southeast Asian journal of tropical medicine and public health, (1995 Dec) 26 (4) 664-8. Journal code: 0266303. ISSN: 0125-1562. Pub. country: Thailand. Language: English.

AB Genotype of three dengue-2 virus strains from Myanmar was determined as genotype II by sequencing 240 nucleotide long fragment across the E/NSI gene junction by the **primer** extension dideoxy chain termination method, applying direct sequencing of the **PCR** product. These strains were isolated from a dengue shock syndrome (DSS) patient and two patients with dengue hemorrhagic fever (DHF) grade 1, in Yangon (Rangoon), Myanmar (Burma), in 1987. Sequence homology of all three strains were highest (96%) to New Guinea C strain (genotype II), lesser homology (93%) to Jamaican 1409 strain (genotype III), and the least homology (91%) to PR 159/SI strain (genotype I). Two DHF strains revealed only 2 nucleotide and 3 nucleotide differences compared with DSS strain, all at the 3rd position of the codons which resulted in silent mutations.

L15 ANSWER 45 OF 65 MEDLINE on STN

97088767. PubMed ID: 8934675. Detection of the disease severity-related molecular differences among new Thai dengue-2 isolates in 1993, based on their structural proteins and major non-structural protein NS1 sequences. Thant K Z; Morita K; Igarashi A. (Department of Virology, Nagasaki University, Japan.) Microbiology and immunology, (1996) 40 (3) 205-16. Journal code: 7703966. ISSN: 0385-5600. Pub. country: Japan. Language: English.

AB We determined the nucleotide sequences of the whole structural protein gene of four new dengue-2 viruses by the **primer** extension dideoxy chain termination method, using multiple cDNA clones for six overlapping gene regions. The nucleotide sequences of the major non-structural protein NS1 gene of these viruses were also determined by direct sequencing of the reverse-transcription **polymerase chain reaction** products. These viruses were isolated from dengue patients with different clinical severities in Nakhon Phanom, Northeastern Thailand in 1993. The results were compared with the sequences of prototype New Guinea C strain and other reference strains. All four viruses revealed highest homology to New Guinea C strain. The homology between each of the four strains and New Guinea C strain varies from 95.09% to 95.29% in its nucleotide sequences, and from 97.24% to 97.78% in its amino acid sequences covering all structural proteins and NS1 protein. The PreM region shows the highest divergence (6.59% to 7.32%) in its nucleotide sequence, whereas C protein is most highly conserved (only 1.75% to 2.63% divergence). Our data showed that there are certain molecular differences in the genomic structure of these four new isolates, which indicate the possibility that these changes are related with the virulence of the virus strains.

L15 ANSWER 46 OF 65 MEDLINE on STN

96038349. PubMed ID: 8530570. Detection of dengue viral RNA by microplate hybridization. Ruiz B H; Zamora M P; Liu S. (Department of Molecular Biology, National University of Mexico, Ciudad Universitaria, Mexico, D.F.) Journal of virological methods, (1995 Aug) 54 (2-3) 97-108. Journal code: 8005839. ISSN: 0166-0934. Pub. country: Netherlands. Language: English.

AB **Dengue virus** infection is a major public health problem throughout tropical countries. In endemic areas, dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS) are common complications resulting in death. However, serological confirmation of dengue-related illness is often complicated and time-consuming. Detection of dengue viruses in clinical or field samples usually depends on virus isolation in susceptible cell lines or in mosquitoes, followed by viral protein identification using polyclonal or monoclonal antibodies. The increasing incidence of **dengue virus** infections has prompted increased efforts to develop rapid and reliable diagnostic techniques. A simple microplate hybridization method was developed for identification of viral RNA. Microplate hybridization is simpler than enzyme-linked immunosorbent assay and has several advantages over the conventional dot-blot hybridization method: (1) radioisotopes are not necessary; (2) synthetic oligonucleotide for the probe is not needed; (3) the time required for washing of the solid phase is greatly reduced; and (4) baking is eliminated. The results show that this procedure is sensitive, rapid and easy to perform.

L15 ANSWER 47 OF 65 MEDLINE on STN

95363978. PubMed ID: 7637011. BHK cell proteins that bind to the 3' stem-loop structure of the West Nile virus genome RNA. Blackwell J L; Brinton M A. (Department of Biology, Georgia State University, Atlanta 30303, USA.) Journal of virology, (1995 Sep) 69 (9) 5650-8. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language:

AB The first 83 3' nucleotides of the genome RNA of the flavivirus West Nile encephalitis virus (WNV) form a stable stem-loop (SL) structure which is followed in the genome by a smaller SL. These 3' structures are highly conserved among divergent flaviviruses, suggesting that they may function as cis-acting signals for RNA replication and as such might specifically bind to cellular or viral proteins. Cellular proteins from uninfected and WNV-infected BHK-21 S100 cytoplasmic extracts formed three distinct complexes with the WNV plus-strand 3' SL [(+)3'SL] RNA in a gel mobility shift assay. Subsequent competitor gel shift analyses showed that two of these RNA-protein complexes, complexes 1 and 2, contained cell proteins that specifically bound to the WNV (+)3'SL RNA. UV-induced cross-linking and Northwestern blotting analyses detected WNV (+)3'SL RNA-binding proteins of 56, 84, and 105 kDa. When the S100 cytoplasmic extracts were partially purified by ion-exchange chromatography, a complex that comigrated with complex 1 was detected in fraction 19, while a complex that comigrated with complex 2 was detected in fraction 17. UV-induced cross-linking experiments indicated that an 84-kDa cell protein in fraction 17 and a 105-kDa protein in fraction 19 bound specifically to the WNV (+)3'SL RNA. In addition to binding to the (+)3'SL RNA, the 105-kDa protein bound to the SL structure located at the 3' end of the WNV minus-strand RNA. Initial mapping studies indicated that the 84- and 105-kDa proteins bind to different regions of the (+)3'SL RNA. The 3'-terminal SL RNA of another flavivirus, **dengue virus** type 3, specifically competed with the WNV (+)3'SL RNA in gel shift assays, suggesting that the host proteins identified in this study are flavivirus specific.

L15 ANSWER 48 OF 65 MEDLINE on STN
95256390. PubMed ID: 7738139. Rapid, single-step RT-PCR typing of dengue viruses using five NS3 gene **primers**. Seah C L; Chow V T; Tan H C; Can Y C. (Department of Microbiology, Faculty of Medicine, National University of Singapore.) Journal of virological methods, (1995 Feb) 51 (2-3) 193-200. Journal code: 8005839. ISSN: 0166-0934. Pub. country: Netherlands. Language: English.

AB In order to detect and type dengue viruses in serum specimens, four type-specific downstream **primers** were designed for use with a consensus upstream **primer** in a reverse transcription and **polymerase chain reaction** (RT-PCR) assay. RT-PCR using these five **primers** amplified NS3 gene fragments of diagnostic sizes of 169, 362, 265 and 426 base pairs for **dengue virus** types 1, 2, 3 and 4, respectively, but not for Japanese encephalitis, Kunjin and yellow fever viruses. The conventional two-step RT-PCR procedure was simplified by combining RT and PCR in a single-step format with a "hot start". This RT-PCR protocol was applied successfully to **dengue virus**-spiked serum and dengue patient serum samples, and could detect as few as one PFU of **dengue virus**. This assay offers a rapid, specific and sensitive molecular technique for the simultaneous detection and typing of dengue viruses.

L15 ANSWER 49 OF 65 MEDLINE on STN
95247869. PubMed ID: 7730437. Monitoring the cDNA synthesis of dengue-2 virus by RT PCR. Liu H S; Tzeng H C; Chen C C. (Department of Microbiology, National Cheng Kung University, Tainan, Taiwan, R.O.C.) Journal of virological methods, (1995 Jan) 51 (1) 55-9. Journal code: 8005839. ISSN: 0166-0934. Pub. country: Netherlands. Language: English.

AB Reverse transcriptase **polymerase chain reaction** (RT PCR) was utilized to observe the complementary DNA (cDNA) synthesis from the 10723 base dengue-2 virus template by in vitro reverse transcription. The PCR product amplified from 5'-end of the genome (PCR **primers** N1A-E1) indicates the completeness of the cDNA synthesis because the cDNA **primer** D8B was located at 3'-end and the cDNA synthesized encompassed the entire RNA template. The integrity of the **dengue virus** RNA was also determined by the cDNA **primer** extension from 3'-end and the PCR amplification at various regions from 5'-end of the RNA template. In conclusion, RT PCR could be applied to monitor cDNA synthesis by in vitro reverse transcription and measure the integrity of viral RNA template. Moreover, it is demonstrated that at least 10.7 kilobases of the cDNA could be synthesized from dengue-2 virus RNA by in vitro reverse transcription.

L15 ANSWER 50 OF 65 MEDLINE on STN
95213156. PubMed ID: 7698880. Comparative analysis of NS3 sequences of temporally separated dengue 3 virus strains isolated from southeast Asia. Chow V T; Seah C L; Chan Y C. (Department of Microbiology, Faculty of Medicine, National University of Singapore, Kent Ridge.) Intervirology, (1994) 37 (5) 252-8. Journal code: 0364265. ISSN: 0300-5526. Pub. country: Switzerland. Language: English.

AB By a combination of PCR and direct-cycle sequencing using consensus **primers**, we analyzed approximately 400-bp fragments within the NS3 genes of twenty-one **dengue virus** type 3 strains isolated from five neighboring Southeast Asian countries at different time intervals from 1956 to 1992. The majority of base disparities were silent mutations,

conservation of the NS3 gene. Phylogenetic trees constructed on the basis of these nucleotide differences revealed distinct but related clusters of strains from the Philippines, Indonesia, and strains from Singapore and Malaysia of the 1970s and early 1980s, while the Thai cluster was relatively more distant. This genetic relationship was compatible with that proposed by other workers who have studied other dengue 3 virus genes such as E, M and prM. However, we observed that the more recent, epidemic-associated dengue 3 strains from Singapore and Malaysia of the late 1980s and early 1990s were more closely related to the Thai cluster, implying their evolution from the latter, and emphasizing the importance of viral spread via increasing travel within the Southeast Asian area and elsewhere. Nucleotide sequence analysis of the NS3 genes of dengue viruses can serve to advance the understanding of the epidemiology and evolution of these viruses.

L15 ANSWER 51 OF 65 MEDLINE on STN

95208980. PubMed ID: 7694966. Partial nucleotide and amino acid sequences of the envelope and the envelope/nonstructural protein-1 gene junction of four dengue-2 virus strains isolated during the 1981 Cuban epidemic. Guzman M G; Deubel V; Pelegrino J L; Rosario D; Marrero M; Sariol C; Kouri G. (Virology Department, Pedro Kouri Tropical Medicine Institute, Havana, Cuba.) The American journal of tropical medicine and hygiene, (1995 Mar) 52 (3) 241-6. Journal code: 0370507. ISSN: 0002-9637. Pub. country: United States. Language: English.

AB In 1981, an epidemic of dengue hemorrhagic fever (DHF) caused by dengue-2 virus occurred in Cuba. This was the first DHF epidemic reported in the Western Hemisphere. In this study, we have analyzed four dengue-2 Cuban strains for two short genomic fragments: one on the envelope (E) glycoprotein and one at the E/nonstructural protein-1 (NS1) gene junction. The E segment of these 1981 Cuban isolates were more closely related to older dengue-2 virus strains such as New Guinea C 1944, Thailand 1964, Sri Lanka 1968, and Burma 1976 than to more recent isolates of this virus from Jamaica and Vietnam. More than 9% of the divergence with strains isolated from Jamaica and Vietnam was observed at the E/NS1 gene junction. One nucleotide change was observed between the first strain isolated during the epidemic and the rest of the Cuban strains. This mutation induced a nonconserved amino acid change from phenylalanine to leucine at position 43 that was not observed in any of the other strains with which it was compared.

L15 ANSWER 52 OF 65 MEDLINE on STN

95126778. PubMed ID: 7826210. Protective efficacy in mice of a secreted form of recombinant dengue-2 virus envelope protein produced in baculovirus infected insect cells. Delenda C; Frenkiel M P; Deubel V. (Unite des Arbovirus et Virus des Fievres Hemorragiques, Institut Pasteur, Paris, France.) Archives of virology, (1994) 139 (1-2) 197-207. Journal code: 7506870. ISSN: 0304-8608. Pub. country: Austria. Language: English.

AB We constructed a recombinant baculovirus encoding a dengue (DEN)-2 virus envelope glycoprotein truncated of 102 amino acids (aa) at its C-terminus (D2E delta 102). The production, processing and transportation of the recombinant protein in baculovirus-infected *Spodoptera frugiperda* (Sf9) cells and its immunogenic properties in mice were compared to those of a previously characterized recombinant DEN-2 E-protein with a 71aa C-terminal truncation (D2E delta 71). Both proteins were transported through the Golgi complex and their N-oligosaccharides of the high mannose type were processed to the complex mannose type. D2E delta 102 transited to the plasma membrane and was secreted whereas D2E delta 71 presumably remained associated with the plasma membrane. The reactivities of the recombinant proteins with neutralizing monoclonal antibodies were similar. Both intracellular and extracellular D2E delta 102 induced neutralizing antibodies in mice and were thus immunogenic. The level of protective immunity to DEN-2 virus encephalitis challenge in mice vaccinated with intracellular D2E delta 102 (80%, $p < 0.01$) was lower than that induced with D2E delta 71 (90%, $P < 0.001$). Sixty-eight percent ($P < 0.001$) of mice vaccinated with 5 micrograms of extracellular D2E delta 102 protein were protected against lethal challenge.

L15 ANSWER 53 OF 65 MEDLINE on STN

95077054. PubMed ID: 7985746. The first epidemic of dengue hemorrhagic fever in French Guiana. Reynes J M; Laurent A; Deubel V; Telliam E; Moreau J P. (Centre de Reference pour la Dengue et la Fievre Jaune, Institut Pasteur de la Guyane, Cayenne, French Guiana.) American journal of tropical medicine and hygiene, (1994 Nov) 51 (5) 545-53. Journal code: 0370507. ISSN: 0002-9637. Pub. country: United States. Language: English.

AB From July 1991 to October 1992, an outbreak of dengue spread into the main urban areas of French Guiana, where 90% of the country's 114,808 inhabitants live. In mid-July 1991 dengue-2 virus was identified as being responsible for most cases, while dengue-1 virus was rarely isolated and circulated at a low level. The number of dengue cases during this period was unknown because there was no clinically based dengue surveillance system. The only available data were for the number of suspected cases as

to a laboratory for dengue diagnosis. Eight hundred forty-seven of the 2,948 suspected cases were diagnosed in the laboratory as dengue cases. Six fatal cases were reported. This outbreak was marked by the appearance of the first clinical cases of dengue hemorrhagic fever (DHF) in French Guiana. Forty cases met the World Health Organization definition of clinical DHF: 32 were grade II, seven were grade III, and one was grade IV and fatal. Eighteen cases were confirmed in the laboratory and 12 were probable; there was no proof of the dengue etiology for the remaining patients.

L15 ANSWER 54 OF 65 MEDLINE on STN

95023164. PubMed ID: 7937125. Ligation of multiple DNA fragments through uracil-DNA glycosylase generated ligation sites. Liu H S; Tzeng H C; Liang Y J; Chen C C. (Department of Microbiology and Immunology, National Cheng Kung University, Tainan, Republic of China.) Nucleic acids research, (1994 Sep 25) 22 (19) 4016-7. Journal code: 0411011. ISSN: 0305-1048. Pub. country: ENGLAND: United Kingdom. Language: English.

L15 ANSWER 55 OF 65 MEDLINE on STN

94147188. PubMed ID: 8313184. Application of reverse transcription-polymerase chain reaction in rapid diagnosis and serotype identification of dengue virus infections. Li G. (Department of Infectious Disease, Sun Yat-sen University of Medical Sciences, Guangzhou.) Zhonghua yi xue za zhi, (1993 Oct) 73 (10) 605-8, 638. Journal code: 7511141. ISSN: 0376-2491. Pub. country: China. Language: Chinese.

AB RT-PCR was developed for the amplification of partial E genome fragment from four dengue serotypes. Of the six oligonucleotide primers designed one was shared by dengue virus type 1 and type 2, and one by type 3 and type 4. Each of the other four primers was type specific. Amplified products with 240, 150, 333 and 421 bp, respectively were identified by electrophoresis on 2% agarose gel and digestion with restriction enzyme Hind III. RT-PCR can detect dengue viral RNA from at least 5TCID50 virus, which was confirmed by detection of serial dilutions of culture supernatants. RT-PCR was also applied to serum samples from 60 acute patients. The findings showed that RT-PCR was of the same specificity as isolation of virus, followed by indirect fluorescent antibody tests. RT-PCR was more sensitive than cell culture and can be used for the rapid diagnosis and serotype identification of dengue virus infections.

L15 ANSWER 56 OF 65 MEDLINE on STN

94058669. PubMed ID: 8240006. Use of NS3 consensus primers for the polymerase chain reaction amplification and sequencing of dengue viruses and other flaviviruses. Chow V T; Seah C L; Chan Y C. (Department of Microbiology, Faculty of Medicine, National University of Singapore, Kent Ridge.) Archives of virology, (1993) 133 (1-2) 157-70. Journal code: 7506870. ISSN: 0304-8608. Pub. country: Austria. Language: English.

AB Consensus primers for the polymerase chain reaction were designed based on conserved motifs within the serine protease and RNA helicase domains encoded by the NS 3 genes of dengue and other flaviviruses. Target fragments of 470 bp were amplified on cDNA templates synthesized from RNAs of dengue types 1, 2, 3, and 4, Japanese encephalitis, Kunjin, and yellow fever viruses using random or specific downstream primers. PCR of oligo(dT)-primed cDNAs from Japanese encephalitis and Kunjin viral RNAs did not yield target bands. As few as 10(3) copies of dengue viral RNA could be detected. Direct DNA sequencing of PCR products of reference strains of dengue 2 (NGC), Kunjin (MRM 61C) and yellow fever (17 D) viruses demonstrated complete concurrence with published data. However, 2 nucleotide differences were observed between our data for dengue 3 H87 strain and the published sequence, resulting in a single amino acid disparity. Differences at 21, 16, and 11 nucleotide positions were noted between dengue 1 Hawaii and S 275/90; dengue 4 H 241 and 814669; Japanese encephalitis Nakayama and JaOArS 982 viral strains, culminating in only 4, 1 and 1 amino acid residue differences, respectively. These amino acid disparities occurred outside putative active sites of the enzymatic domains, emphasizing the important role of the NS3 protein in flaviviral replication. This RNA-PCR consensus primer strategy coupled with DNA sequencing represents a valuable tool for the molecular diagnosis and epidemiology of dengue and other flaviviral infections.

L15 ANSWER 57 OF 65 MEDLINE on STN

93055218. PubMed ID: 1385464. A simple and rapid method of preparing large fragments of dengue virus cDNA from replicative-form RNA using reverse transcriptase and PCR. Chen W; Qu X; Maguire T. (Microbiology Department, University of Otago, Dunedin, New Zealand.) Journal of virological methods, (1992 Sep) 39 (1-2) 197-206. Journal code: 8005839. ISSN: 0166-0934. Pub. country: Netherlands. Language: English.

AB A method is described for cloning large fragments (1.5 kb to 2 kb) of dengue virus cDNA from replicative-form viral RNA. Aedes albopictus cells (C6/36 clone) infected with dengue virus contain double-stranded, replicative-form RNA molecules which were used as a

sequence homologous to regions of the genome at or near the 3' end of the gene being studied. The product was then used as a template for **polymerase chain reaction (PCR)** amplification using the same 3' **primer** and a second which hybridized to a region at the 5' end of the sequence to be cloned. Both **primers** were engineered to contain specific restriction enzyme cutting sites which enabled the **PCR** product to be cut and cloned directly into plasmids for sequencing and expression studies. We have used this method to construct clones of the envelope glycoprotein gene (E) and the non-structural genes 1 and 2a (NS1/2a) and 3 (NS3) of dengue type 2, Tonga 1974 strain, and E and NS1/2a from dengue type 3, H-87 strain, either as discrete genes or as constructs with long and short leader sequences, with or without anchor sequences. The method could be applied to the cloning of any gene from any flavivirus, directly from infected cell extracts, without the necessity for tedious virus purification steps.

L15 ANSWER 58 OF 65 MEDLINE on STN

92397084. PubMed ID: 1381844. Amplification of dengue 2 virus ribonucleic acid sequence using the **polymerase chain reaction**. Chen H S; Guo H Y; Chen H Y; Liag Y K. (Department of Microbiology, Sun Yat-sen University of Medical Sciences, Guangzhou, People's Republic of China.) Southeast Asian journal of tropical medicine and public health, (1992 Mar) 23 (1) 30-6. Journal code: 0266303. ISSN: 0125-1562. Pub. country: Thailand. Language: English.

AB The **polymerase chain reaction (PCR)** has been adapted to the amplification of dengue type 2 virus (DEN2) nucleic acid sequences. A pair of 20-mer oligonucleotides were designed and synthesized based on conserved sequence blocks of DEN2 strains isolated from different geographical areas. RNA samples were prepared from two DEN2 strains, prototype New Guinea C (NGC) and local isolate Hainan 98 (HN98). The reverse transcription step was performed for cDNA synthesis before the standard **PCR** procedures. The amplified products were fragments about 476 bp in length, corresponding to the upper one third of DEN2 envelope gene (E1 to E476 nt). Specificity of the amplification products was confirmed by "nested" **PCR** using the internal **primers** and by Southern and dot blot hybridization to cloned DEN2 cDNA probes following agarose gel electrophoresis. Further improvement and the potential application of the methods in study of **dengue virus** RNA are discussed.

L15 ANSWER 59 OF 65 MEDLINE on STN

92355728. PubMed ID: 1379606. Direct sequencing of large flavivirus **PCR** products for analysis of genome variation and molecular epidemiological investigations. Lewis J G; Chang G J; Lanciotti R S; Trent D W. (Division of Vector-Borne Infectious Diseases, Centers for Disease Control, Fort Collins, CO 80522.) Journal of virological methods, (1992 Jul) 38 (1) 11-23. Journal code: 8005839. ISSN: 0166-0934. Pub. country: Netherlands. Language: English.

AB The **polymerase chain reaction (PCR)** was used to amplify viral cDNAs from selected regions of dengue genomic RNA by using appropriate 'consensus' **primers**. DNA amplicons containing the structural genes from all 4 dengue serotypes were prepared and directly sequenced using **dengue-virus-specific primers**. This method can characterize reliably flavivirus field isolates at the molecular level without extensive virus propagation and molecular cloning, and will be a valuable tool for molecular epidemiological studies.

L15 ANSWER 60 OF 65 MEDLINE on STN

92340638. PubMed ID: 1634599. Direct sequence analysis of amplified **dengue virus** genomic RNA from cultured cells, mosquitoes and mouse brain. Lee E; Nestorowicz A; Marshall I D; Weir R C; Dalgarno L. (Division of Biochemistry and Molecular Biology, School of Life Sciences, Faculty of Science, Australian National University, Canberra, ACT.) Journal of virological methods, (1992 Jun) 37 (3) 275-88. Journal code: 8005839. ISSN: 0166-0934. Pub. country: Netherlands. Language: English.

AB A method is described for direct sequence analysis of selected regions of **dengue virus** genomic RNA in infected tissues. Using specific **primers**, total high-molecular-weight infected-cell RNA is reverse transcribed to single-stranded (ss) complementary DNA, amplified using the **polymerase chain reaction (PCR)** and sequenced using ssDNA obtained after lambda exonuclease digestion of one strand of the **PCR** product (R.G. Higuchi and H. Oehman, Nucleic Acids Research, 17, 5865, 1989). Sequence data for the envelope protein gene of two dengue-3 virus isolates were obtained using RNA from small numbers (10(5)) of cultured mosquito or monkey kidney cells, from one mg of infected mouse brain and from 1/300th of an infected *Toxorhynchites amboinensis* mosquito. Independent determinations showed that errors occurring during reverse transcription or **PCR** were not represented to a significant degree in the sequence of the amplified DNA. The method does not depend on extensive passaging of virus or large-scale growth to generate material for sequencing and therefore provides a means of obtaining sequence data for unadapted **dengue virus** isolates.

92202358. PubMed ID: 1372617. Rapid detection and typing of dengue viruses from clinical samples by using reverse transcriptase-**polymerase chain reaction**. Lanciotti R S; Calisher C H; Gubler D J; Chang G J; Vorndam A V. (Division of Vector-Borne Infectious Diseases, Centers for Disease Control, Fort Collins, Colorado 80522.) Journal of clinical microbiology, (1992 Mar) 30 (3) 545-51. Journal code: 7505564. ISSN: 0095-1137. Pub. country: United States. Language: English.
- AB We report on the development and application of a rapid assay for detecting and typing dengue viruses. Oligonucleotide consensus **primers** were designed to anneal to any of the four **dengue virus** types and amplify a 511-bp product in a reverse transcriptase-**polymerase chain reaction (PCR)**. First, we produced a cDNA copy of a portion of the viral genome in a reverse transcriptase reaction in the presence of **primer** D2 and then carried out a standard **PCR** (35 cycles of heat denaturation, annealing, and **primer** extension) with the addition of **primer** D1. The resulting double-stranded DNA product of the RT-**PCR** was typed by two methods: dot blot hybridization of the 511-bp amplified product to **dengue virus** type-specific probes or a second round of **PCR** amplification (nested **PCR**) with type-specific **primers**, yielding DNA products the unique sizes of which were diagnostic for each **dengue virus** serotype. The accumulated data demonstrated that dengue viruses can be accurately detected and typed from viremic human serum samples.
- L15 ANSWER 62 OF 65 MEDLINE on STN
92113574. PubMed ID: 1339466. Envelope protein sequences of **dengue virus** isolates TH-36 and TH-Sman, and identification of a type-specific genetic marker for dengue and tick-borne flaviviruses. Shiu S Y; Jiang W R; Porterfield J S; Gould E A. (Department of Microbiology, University of Hong Kong.) The Journal of general virology, (1992 Jan) 73 (Pt 1) 207-12. Journal code: 0077340. ISSN: 0022-1317. Pub. country: ENGLAND: United Kingdom. Language: English.
- AB Complementary DNAs were synthesized from the envelope protein genes of two isolates of **dengue virus** (TH-36 and TH-Sman, previously suggested as possible **dengue virus** type 5 and **dengue virus** type 6 respectively) and amplified by the **polymerase chain reaction** using sense and antisense **primers** designed from conserved **dengue virus** gene sequences. The amplified cDNA clones were sequenced in both directions by double-stranded dideoxynucleotide sequencing. Alignment with published **dengue virus** sequences enabled us to assign these viruses accurately to classified serotypes, confirming that TH-36 and TH-Sman are strains of **dengue virus** type 2 and **dengue virus** type 1 respectively. Amino acid changes between the proteins encoded by these two isolates and strains of their respective serotypes may account for the significant antigenic differences observed during previous serological typing of these viruses. Moreover, sequence alignment of flavivirus envelope proteins revealed a hypervariable region, within which members of the dengue and tick-borne virus antigenic complexes show unique peptide sequences. This type-specific hypervariable domain may be useful as a genetic marker for typing dengue and tick-borne flaviviruses.
- L15 ANSWER 63 OF 65 MEDLINE on STN
92059660. PubMed ID: 1951850. Sensitivity and specificity of a universal **primer** set for the rapid diagnosis of **dengue virus** infections by **polymerase chain reaction** and nucleic acid hybridization. Henchal E A; Polo S L; Vorndam V; Yaemsiri C; Innis B L; Hoke C H. (Department of Virus Diseases, Walter Reed Army Institute of Research, Washington, DC.) American journal of tropical medicine and hygiene, (1991 Oct) 45 (4) 418-28. Journal code: 0370507. ISSN: 0002-9637. Pub. country: United States. Language: English.
- AB A set of sense and anti-sense oligomeric DNA **primers**, degenerate in the third "wobble" base position of codons so as to match all known **dengue virus** sequences, was evaluated as universal **primers** in a **polymerase chain reaction (PCR)** assay for the rapid diagnosis of **dengue virus** infections. Virus-specific complementary DNA (cDNA) was prepared by reverse transcription (RT) of total RNA extracted from serum. Amplified cDNA was identified by nucleic acid hybridization with four serotype-specific, oligomeric DNA probes. Using sera from patients admitted with dengue fever, RT/**PCR** followed by nucleic acid hybridization using radiolabeled probes was 68% sensitive (50/74; 95% confidence interval [CI] = 57-78%) and 100% specific. Chemiluminescent detection of hybridized products was 62% sensitive (26/42; 95% CI = 46-75%). Using specimens from which a virus isolate had been obtained, RT/**PCR** followed by nucleic acid hybridization with radiolabeled probes was 80% sensitive (40/50; 95% CI = 69-91%) and 100% specific. The results suggest that RT/**PCR** using degenerate **primers** is a sensitive and specific method for the detection of dengue viruses in clinical specimens.
- L15 ANSWER 64 OF 65 MEDLINE on STN
92042627. PubMed ID: 1682341. Rapid identification of **dengue virus** serotypes by using **polymerase chain reaction**. Morita K; Tanaka M; Igarashi A. (Department of Virology, Nagasaki University, Japan.) Journal of clinical microbiology, (1991 Oct) 29 (10) 2107-10. Journal code:

AB Four primer pairs were selected on the basis of the published sequence data of four **dengue virus** serotypes so that each unique target sequence size could be amplified for each serotype by **polymerase chain reaction**. The procedure consists of (i) RNA preparation, (ii) reverse transcription, and (iii) **polymerase chain reaction**, all of which could be completed within 2 h in a single tube for each specimen. The amplified sequence size revealed by ethidium bromide-stained agarose gel electrophoresis was unique for each serotype, using infected culture fluid of isolates from dengue fever or dengue hemorrhagic fever patients in Thailand, Indonesia, and the Philippines as well as from prototype viruses, thus facilitating simultaneous identification and typing.

L15 ANSWER 65 OF 65 MEDLINE on STN

91201502. PubMed ID: 2086596. Identification of dengue sequences by genomic amplification: rapid diagnosis of **dengue virus** serotypes in peripheral blood. Deubel V; Laille M; Hugnot J P; Chungue E; Guesdon J L; Drouet M T; Bassot S; Chevrier D. (Institut Pasteur, Laboratoire des Arbovirus, Paris, France.) Journal of virological methods, (1990 Oct) 30 (1) 41-54. Journal code: 8005839. ISSN: 0166-0934. Pub. country: Netherlands. Language: English.

AB **Polymerase chain reaction (PCR)** was developed for the in vitro amplification of **dengue virus** RNA via cDNA. A fraction of the N-terminus gene of the envelope protein in the four dengue serotypes was amplified using synthetic oligonucleotide **primer** pairs. Amplified products were cloned and used as dengue type-specific probes in gel electrophoresis and dot-blot hybridization. We detected and characterized **dengue virus** serotypes in blood samples by the three-step procedure DNA-PAH consisting in cDNA priming (P), DNA amplification (A) and hybridization (H) using specific non-radiolabelled probes. Our findings showed that DNA-PAH was more rapid and sensitive in the identification of the infecting serotype than the mosquito cell cultures. Moreover, the failure of cultures to detect virus particles in sera containing few copies of viral genome or anti-dengue antibodies justified the approach of DNA-PAH to the dengue identification in clinical specimens.

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L1 12 S E3
L2 1163 S (DENGUE VIRUS)
L3 727 S L2 AND (PCR OR POLYMERASE CHAIN REACTION)
L4 178 S L3 AND (PRIMER?/CLM OR OLIGONUCLEOTIDE?/CLM)
L5 112 S L4 AND AY<2003

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E WANG WEI KUNG/IN

E WANG W K/IN

L6 11 S E3
L7 213 S (DENGUE VIRUS)
L8 14 S L7 AND (PCR OR POLYMERASE CHAIN REACTION)
L9 14 S L8 NOT L6

FILE 'MEDLINE' ENTERED AT 23:01:45 ON 03 FEB 2006

E WANG W K/AU

L10 60 S E3
L11 1 S L10 AND DENGUE
L12 2828 S (DENGUE VIRUS)
L13 329 S L12 AND (PCR OR POLYMERASE CHAIN REACTION)
L14 78 S L13 AND (PRIMER? OR OLIGONUCLEOTIDE?)
L15 65 S L14 AND PY<2003

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